STORAGE STABILITY OF ASEPTICALLY PACKAGED SINGLE STRENGTH ORANGE JUICE AND ORANGE DRINKS

BY

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IN MEMORY OF MY FATHER

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LIST OF ABBREVIATIONS

AA	ascorbic acid
AER	aerobic
ANA	anaerobic
ANOVA	analysis of variance
a_w	water activity
Cpd	compound
DHAA	dehydroascorbic acid
DKGA	diketogulonic acid
DP	3-deoxy-L-pentosone
FA	furoic acid
FDA	Food and Drugs Administration
HFCS	high fructose corn syrup
HPLC	high performance liquid chromatography
MMT	million metric tons
MT	million tons
OJD	orange juice deaerated
OJND	orange juice nondeaerated
OPDA	orthophenylenediamine
RDA	recommended daily allowances
SD	standard deviation
SSOJ	single strength orange juice

Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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Nonenzymatic browning of single strength orange juice, and synthetic orange drinks containing 10% orange juice and various combinations of ascorbic acid, amino acids (aspartic acid, arginine, and 4-aminobutyric acid), sugars, citric acid and potassium citrate has been studied under aerobic and anaerobic conditions. The juice and the drinks were aseptically packaged and stored at 75°F for up to 20 weeks. In the presence of oxygen, ascorbic acid was found to be the most reactive constituent in the darkening of orange drinks. The presence of amino acids at the high level (1.26%) increased significantly the rate of ascorbic acid degradation and the rate and extent of browning pigments formation. However, reducing the amino acids level from 0.66 to 0.06% had no significant effect on the browning of orange drinks stored under anaerobic conditions, but did significantly affect browning when samples were stored aerobically. The study also showed that the storage of aseptically packaged orange

juice in anaerobic jars, as compared to aerobic storage, resulted in higher ascorbic acid retention. However, there was no significant difference in the sensory evaluation, nor in the amino acids concentrations after 16 week storage at 75°F.

In addition, a high-performance liquid chromatography (HPLC) procedure has been developed for the rapid and simultaneous estimation of ascorbic and dehydroascorbic acids in fresh fruits and vegetables. Isocratic separation of these components was accomplished by anion-exchange chromatography using acetonitrile:0.05M KH_2PO_4 (75:25, v/v) as eluant. The concentration of ascorbic acid was determined by monitoring its absorbance at 254 nm, while dehydroascorbic acid detection was achieved by fluorescence as a result of post-column derivatization involving the condensation of dehydroascorbic acid with o-phenylenediamine, forming a highly fluorescent quinoxaline derivative. The procedure allows detection of both forms of vitamin C at levels well below those usually found in orange juice, and was used to follow the rate of change of ascorbic acid into dehydroascorbic acid in orange juice and orange drinks during storage.

INTRODUCTION

Citrus products during processing and storage at room temperature are susceptible to a number of deteriorative reactions, which result in the development of off-flavor. Such off-flavor is generally accompanied by other changes, in particular, browning of the product and loss of nutritional value. This type of discoloration called nonenzymatic browning is one of the most disturbing problems for citrus industries. It is the main reason for the reduction in commercial value and consumer rejection of citrus products, and has been the subject of research for many years. Many different types of reactions lead to the discoloration of the product at moderate temperatures. This change in color may occur through formation of dark pigments by the breaking down of certain constituents such as ascorbic acid, by reaction between some constituents present in the juice product or by reaction between some constituents of the juice with oxygen in the air.

Factors which can influence the nature of the degradation mechanism include temperature, oxygen, amino acids, metal catalysts, pH, and sugar concentration. Various hypotheses have been developed to explain the mechanism of browning in citrus juices. Among these, ascorbic acid degradation is thought to be the major pathway responsible for browning in citrus products.

However, in spite of the many reviews of the subject, there has been no comprehensive organization of the reactions involved. The work reported is contradictory in nature, and there is still a lack of understanding concerning the fundamental factors involved in the deterioration of packaged orange juice.

It is not known whether decomposition of ascorbic acid alone or decomposition in the presence of sugars and amino acids of orange juice is more important than the Maillard reaction in browning. A clear knowledge of the reactions involved in this deterioration and the roles played by the various constituents of the juice is necessary before an entirely satisfactory method for controlling nonenzymatic browning in citrus can be realized.

In view of this lack of understanding it was decided to carry out this research project to study the roles of ascorbic acid concentration, amino acids concentration, juice concentration, oxygen concentration, storage and processing conditions, and storage time in the browning of aseptically packaged orange juice and orange drinks containing various combinations of ascorbic acid, amino acids, sugars, citric acid and potassium citrate. In addition, the development of an analytical procedure to follow the rate of change of ascorbic acid into dehydroascorbic acid in orange juice and orange drinks during storage using the HPLC technique was investigated.

LITERATURE REVIEW

A Brief History of Citrus Distribution

Citrus trees first appeared in Chinese gardens during the 13th to 16th centuries. From this area the Arab traders propagated the fruits progressively with their conquests of the Middle East, Near East, North Africa and Spain. The fruits were introduced into North America from the Canary Islands by Christopher Columbus during his second voyage in 1493 (Cooper and Chapot, 1977). The exact date of the introduction of citrus trees into Florida is not known. Ziegler and Wolfe (1961) pointed out that oranges were likely brought into Florida at the time the colony at St. Augustine was established in 1565. Seeds were probably then scattered throughout the State by Indians.

Citrus Production in Florida

The first recorded citrus crop was noted in a report by the Governor of St. Augustine in 1579, but commercial production and trade did not begin to develop on a large scale until after the Civil War. In 1886-87, the U.S.Department of Agriculture reported a total citrus production of 1.26 million boxes (Figure 1). The "Great Freeze" of 1894-95 almost destroyed the entire citrus industry of the state. It was not until 1903-04, that this level was reached again. Since that time the volume has steadily increased to reach a maximum of 283.6 million boxes in 1979-80 (Fla. Crop and Livestock Reporting Ser. Citrus Summary, 1985).

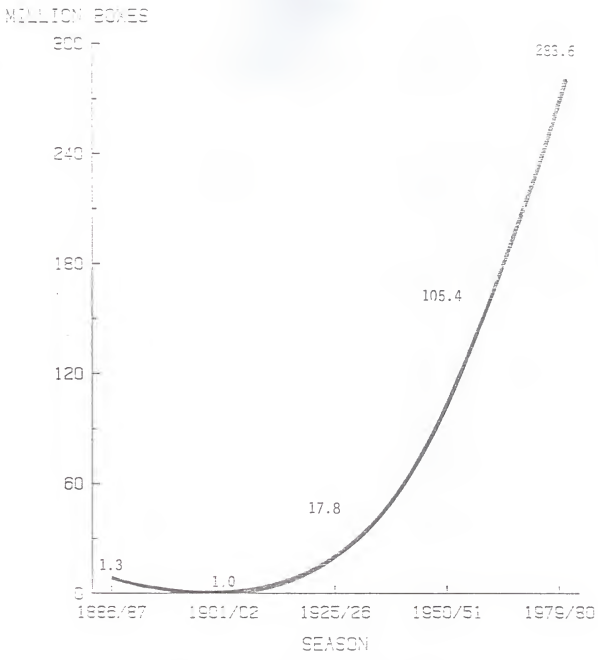

Figure 1. Florida Citrus Production

As the decade of the sixties began, Florida's dominance of the nation's citrus became more apparent, and in the 1983-84 season Florida accounted for more than 69% of the U.S. production of citrus fruits (Figure 2 and Table 1).

Even though the industry suffered crippling natural disasters ranging from winter freezes to canker, Florida's 1984-85 citrus crop has been valued at a record $1.04 billion in a preliminary report (Citrus Valued at Record $1.4 billion, 1985). Although the amount of oranges harvested for 1984-85, 103.9 million boxes (4.6MT), is the smallest yield since 1967-68, higher prices pushed the value to the record level. The total citrus harvest, including oranges, grapefruit, lemons and limes was 158.9 million boxes (7MT). The 1979-80 harvest held the previous record high of $ 1.03 billion, according to the Florida Crop and Livestock Reporting Service of Orlando. The latest report issued by the service shows a 23% increase in value over the 1983-84 harvest of $ 849 million.

Orange production increased from about 2.5 million tons in 1963-64 to over 8 million tons in the 1975-76 season, and presented 78% of the U.S. total production (Figure 3 and Table 2).

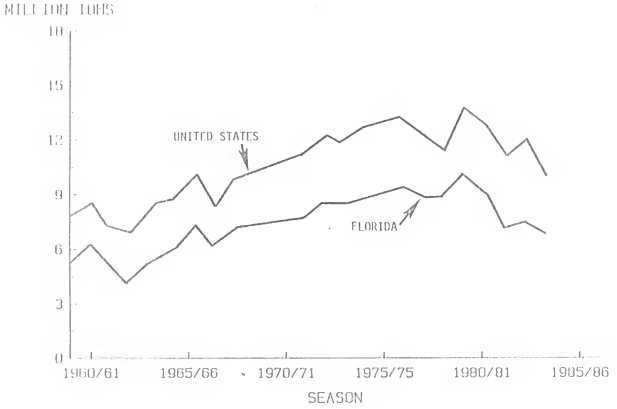

Figure 2. Principal Citrus Fruits: Production for the United States and Florida, Crop Years 1960-61 through 1983-84

Table 1

Principal Citrus Fruits: Production for the United States and Florida, Crop Years 1964-65 through 1983-84 (1,000 Tons)

Year	United States	Florida	%
1964-65	7,633	5,480	71.8
1965-66	8,768	6,242	71.2
1970-71	11,919	8,786	73.7
1975-76	14,788	10,943	74.0
1980-81	15,105	10,470	69.3
1982-83	13,608	8,513	62.6
1983-84	10,741	7,436	69.2

Source: Adapted from Fla. Crop and Livestock Reporting Serv. (1985).

TABLE 2

Oranges: Production for United States and Florida, Crop Years 1960-61 through 1983-84

Crop year	Production: 1,000 tons		%
	United States	Florida	
1963-64	3,732	2,469	66
1965-66	5,812	4,316	74
1970-71	8,205	6,402	78
1975-76	10,493	8,154	78
1980-81	10,487	7,758	74
1982-83	9,519	6,282	66
1983-84	7,238	5,252	73

Source: Adapted from Fla. Crop and Livestock Reporting Service, (1985).

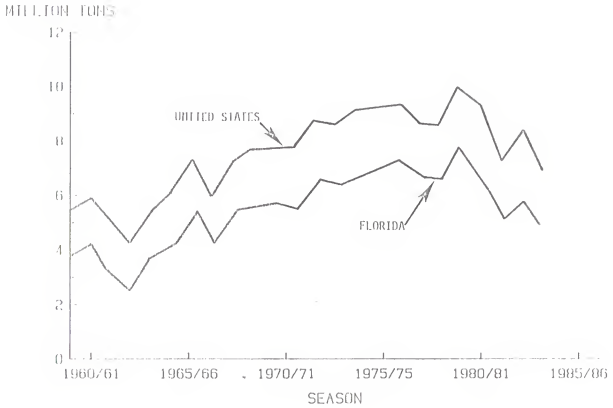

Figure 3. Oranges: Production for United States and Florida, Crop Years 1960-61 through 1983-84

Citrus Demand

The per capita consumption has increased from 22.2 pounds in 1920 to almost 118 ponds in 1980, more than a five-fold increase (Table 3).

Table 3

U.S. Citrus Per Capita Consumption.
Fresh Weight Equivalent (pounds)

Item	1920	1940	1960	1980
Fresh[a]	22.2	52.1	30.7	26.3
Processed	-0-[b]	10.4	52.2	91.2
Total	22.2	62.5	82.9	117.5

[a] Excludes lemons and limes.
[b] None reported.

There has been a major shift in the form of the product demand. Per capita consumption of fresh citrus increased during the early 1900s until the mid-1940s. Since 1940, fresh per capita citrus consumption has declined by about 50%, while processed consumption increased nine-fold (Gunter, 1983). Introduction of canned juice in the 1920's and frozen concentrate in the mid 40' are the major factors contributing to the growth in processed citrus juice demand.

Citrus Processing

National

Before canned orange juice was introduced commercially in 1929, only 1% of the domestic crop was processed. By 1945-47, an average of over 1.4 MMT (million metric tons) of oranges and tangerines were processed with almost the entire volume going into juice related products. This volume represented about 1/3 of the total domestic crop which was reported at 4.6 MMT. By 1959, about 3.5 MMT of oranges and tangerines were processed into juice products representing nearly 64% of the 5.6 MMT crop that year. The percent of processed citrus fruits continued to increase and by 1983-84, processors used 67% of the total citrus crop of 9.74 MMT. They used 74% of the orange production, 53% of the grapefruit and 46% of the lemons compared with 76%, 47%, and 54% respectively, in 1982-83. (Citrus Fruits, 1984).

Florida

In Florida, citrus processing represents the largest food processing industry, producing 73% of the total United States citrus products (Fla. Crop and Livestock Reporting Service, 1985). More than 90% of the Florida's orange crop is processed with 80% utilized for frozen concentrate orange juice, 12% for chilled juice, 3% for canned single strength orange juice; 4% is sold in the fresh market and less than 1% is used in products like sections, salads, and blends. The equivalent of 231 million 45°Brix gallons of concentrated juice were produced during 1979-80 season (Fla. Crop and Livestock Reporting Service, 1980). Table 4 illustrates the upward trend in processed oranges compared to declining trends for fresh oranges in Florida.

Juice Packaging

Juice and juice drink products have been distributed in three forms, shelf-stable products, chilled juices, and frozen concentrates. Single strength citrus juice became available in the early 1930's. During the period from the early 1930's to World War II, commercial flash pasteurization of juice was developed. This allowed processed juices to retain some of their natural fresh aroma and flavor during heat processing. The availabilty of high quality juices in the form of frozen juice concentrates occurred in the 1940's. Prior to the introduction of this new concentrating technology to the United States, only a third of the domestic orange crop was processed into juice. By the late 1940s, with the availability of the improved technology for frozen concentrates, nearly two-thirds of the orange crop was being processed into juice, with the largest percent being used to produce high-quality, frozen orange juice concentrate. The success of these products is attributed to the fact they offer convenience, low cost and are available year round.

However, until recently, the shelf-stable juices and juice drinks have shown a lack of growth in overall consumption compared to juices that were distributed as frozen concentrate or in refrigerated forms. This is because of the higher quality that these "cold" methods of distribution could deliver to the juice and juice products.

Table 4

Florida Oranges: Production, Utilization and Value for Crop Years 1964-65 through 1983-84

Crop year	Utilization of Production			% Processed	On-Tree Value of Production
	1,000 boxes				1,000 dollars
	Total	Fresh	Processed		
1963-64	54,90	11,94	42,96	78	243,935
1965-66	95,90	15,38	80,52	84	155,625
1970-71	142,30	13,96	128,34	90	208,146
1975-76	181,20	11,73	169,47	94	321,449
1980-81	172,40	8,28	164,12	95	697,231
1981-82	125,80	7,62	118,18	94	538,686
1982-83	139,60	10,32	129,28	93	718,420
1983-84	116,70	7,64	109,06	93	578,954

Source: Adapted from Fla. Crop and Livestock Reporting Service, (1985).

The traditional method of processing a shelf-stable juice product involves the hot filling of the juice in cans or glass bottles. This method of processing has some difficulties if one wishes to achieve a high-quality product. In this type of processing and packaging operation, juices are commonly subjected to a severe heat treatment for relatively long periods of time. The usual hot-filling process involves heating and filling a rigid container above 170-180oF (76.7o-82.2oC), followed by a cooling period which requires 10 to 20 minutes for the juice to reach ambient temperature. The severity of this type of processing has often decreased the overall quality of juices processed in this manner. This method of processing has also discouraged the introduction of other quality-improving technologies.

Any potential improvement that could be made in the quality of the juice delivered for bottling or canning was quickly lost due to the severe heat treatment necessary for the hot-filling operation. Another problem affecting the quality of juice products packed in cans is the interaction between the juice and the metal containers. Problems such as the "pick up" of tin by juices in unlined containers contribute to off-flavor of the consumed product. It is also a common practice to select the premium juices for use in chilled and frozen products, and consider shelf-stable juice products as unaffected by long periods of ambient storage, hence disregarding the inherent degradative changes that occur in many fruit juice products when stored at ambient temperature for long periods.

Until recently the most common package style used in processing citrus shelf-stable juice products was the metal container. The tin container guaranteed long shelf life and package integrity. The second most important package, the glass bottle, has the same benefits as the tin can. Both products do not require refrigeration in distribution but the containers added significantly to total freight costs due to their heavy tare weight. The energy crisis of the mid 1970's has dramatically influenced this situation. Although both cans and glass bottles are still used, the trend of the citrus industry is now moving toward low cost, light-weight, limited shelf life packages. The most recent development is the clearance of the "Tetra Brik" package for use in aseptic packaging of liquid products.

Aseptic Processing

Aseptic processing is a processing and packaging technique by which a commercially sterilized product is put into a presterilized, hermetically sealed container in a sterile environment. To produce a commercially sterile product, an aseptic system must meet three basic requirements:

-The product must be sterile.

-The package in which the product will be placed must be sterile.

-The environment in which the product and package will be brought together must be sterile.

Commercial sterility is a term used to denote a product that has been processed in such a manner so that it is free of all contaminants that cause the product to spoil or be a detriment to public health.

Aseptic processing and packaging is not a new technology. The first commercial system in the U.S. was installed in the 1950's for use with non-acid products such as milk. However, since the recent (February 9, 1981) FDA regulation permitting the use of hydrogen peroxide and heat as sterilizing agents for aseptic packaging, the interest in aseptic packaging has increased dramatically. This technique is now applied in several food industries to produce microbially stable products. By using this technology it is possible to minimize the time the product is subjected to the high temperature necessary to obtain commercial sterility, and produce shelf stable

juices that are equal in organoleptic quality to frozen and chilled products, at a more economical price. It is estimated that 1-liter aseptic boxes cost about half as much as cans and 30% as much as bottles (Paper Bottles Are Coming on Stong, 1983). Although some of these savings are surrendered because of the more complicated filling process, it is estimated that the cost of packaging juice concentrate in 8oz "Tetra Brik" boxes is 18% cheaper than filling coventional paper and metal cans (Business Week, January 16, 1984), and since aseptic goods are subjected to a briefer heating treatment during sterilization than canned goods, they have better flavors. In addition a major saving results because they do not require refrigeration during shipment or storage.

However, aseptic processing will not be of commercial importance until chemical stability of citrus products is understood and improved. The limiting factor in the acceptance and eventual success of aseptically packaged citrus juices is the control of chemical changes that are accelerated during ambient temperature distribution and storage. Nonenzymatic browning reaction is believed to be the quickest and most dramatic quality defect to appear during ambient temperature storage (Clegg, 1964; Tatum et al., 1967; and Tatum et al., 1975).

Nonenzymatic Browning

The nonenzymatic browning reaction in foods during processing or on storage has long been recognized as one of the most important problems of fruit preservation, and has been the subject of research for many decades. This reaction refers to the formation of brown

pigments in foods that cause the product to become brownish to black in appearance, and is usually characterized by undesirable changes in flavor, odor, and nutritive value.

In order to study the reaction under controlled conditions, model systems have been widely used, but full details of the physical conditions employed have not always been reported. The work done before 1948 has been reviewed by Stadtman (1948). Since then there have been excellent reviews by Hodge (1953), Reynolds (1963), Burton and McWeeny (1964), Shaw et al. (1977), and Handwerk and Coleman (1986) which mainly covered the reactions between amines and sugars.

Three main theories have been advanced to explain the mechanism of nonenzymatic browning (Stadman, 1948).

- The Maillard or melanoidin condensation theory: According to this theory (the most common) the reaction involves a condensation of amino acids and reducing sugars and gives rise to the formation of dark colored substances.

- The active-aldehyde theory: It proposes that browning involves the decomposition of sugars and sugar acids to furfuraldehydes or similar compounds characterized by having an active carbonyl group, and that these products condense with nitrogen compounds and/or polymerize to form colored substances.

- The ascorbic acid theory: According to this theory the most important precursors to browning are ascorbic acid and related compounds, which upon oxidation yield reactive products that may polymerize or react with nitrogenous constituents of the food to form brown pigments. This third theory seems the most likely to apply to

the conditions pertaining to an acidic product such as orange juice; the concentration of ascorbic acid is relatively high and free amino acids are present to combine with the reactive products resulting from the oxidation of the ascorbic acid and lead to the formation of brown pigments.

Actually all three of the above mechanisms may be involved in the browning of fruit products. Major research efforts have been conducted to prove the first two hypotheses of nonenzymatic browning, as noted in the review by Shaw et al. (1977), but relatively little research has explored the ascorbic acid theory. It is not known whether decomposition of ascorbic acid alone or in the presence of sugars and amino acids of orange juice is more important than the sugar-amino acids reaction in browning. Let us now look at these 3 theories in more detail.

Maillard Reaction

Maillard was the first to study systematically the interaction which occurs initially between amino acids and sugars, and to realize some of their relations to the chemistry of natual products. He found that simple amino acids react on warming with certain sugars, to produce dark-brown products. This explains why the reaction has also been commonly called the browning reaction, nonenzymatic browning, melanoidin formation, or caramelization; and the brown products have been referred to as melanoidins, or humin-like substances (Ellis, 1959).

The interaction of amino acids and sugars falls into two general types. The first is the simple controlled condensation of the

reactants; this leads to compounds which are identifiable as N-substituted glycosylamines or, occasionally their Amadori rearrangement products. The second is the typical Maillard reaction, which leads to a mixture of products of increasing complexity if conditions are favorable.

Reynolds (1970) has outlined the major features of the initial stages of the carbonyl-amine reaction. At the first step, an aldose or ketose sugar reacts with a primary or secondary amine to form a glycosylamine, and the reaction is reversible (Figure 4).

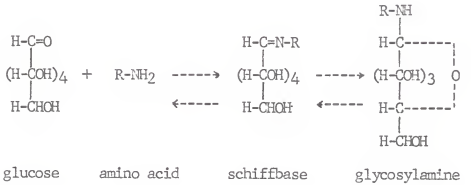

Figure 4. Initial Stage of the Carbonyl-Amine Reactions

The role of water is important in determining the yield of glycosylamine. At low water content there is substantial formation of this compound, (Shallenberger and Birch, 1975); therefore, carbonyl-amine browning is believed to be a significant pathway for browning in dried and concentrated foods. The probable mechanism for the formation of glycosylamine is the addition of the amine to the carbonyl group of the open-chain form of the sugar, followed by

elimination of a molecule of water and subsequent ring closure (Hodge, 1976).

The next step is the Amadori rearrangement which involves the protonation of the nitrogen atom at C-1 (Figure 5). This rearrangement takes place in either acid or basic solutions.

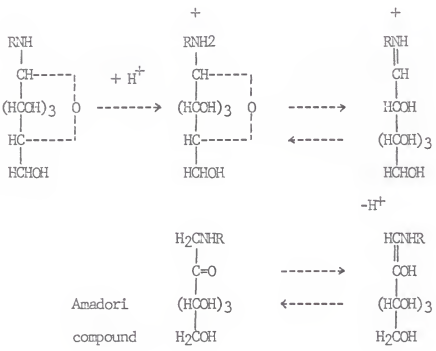

Figure 5. Amadori Rearrangement

The second stage in carbonyl-amine reactions is based on the degradation and dehydration of the Amadori compound, which can occur through two major pathways. The major branch leads from the 1,2-eneaminol of the Amadori compound to hydroxymethyl furfuraldehyde (Figure 6). The amino acid may be retained in some molecules throughout the dehydration reactions of this pathway.

This pathway is the major pathway for the production of brown color in foods. The minor branch which probably represents less than 5% of the total sugar decomposition (Hodge and Osman, 1976) begins with the 2,3-enediol of the Amadori compound; then the amino acid is totally eliminated (Figure 7). The degradation compounds formed seem to be important in the production of flavor.

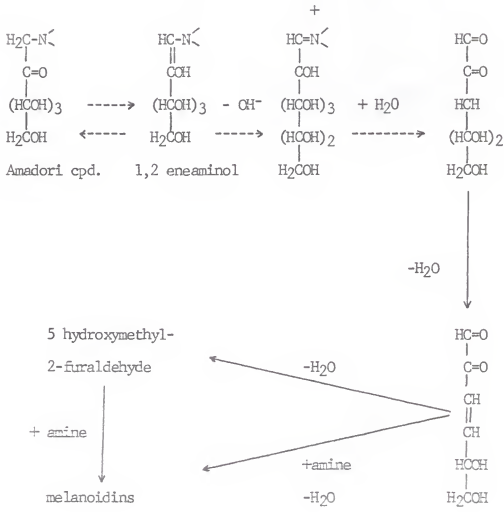

Figure 6. Major Pathway for Carbonyl-Amine Reactions

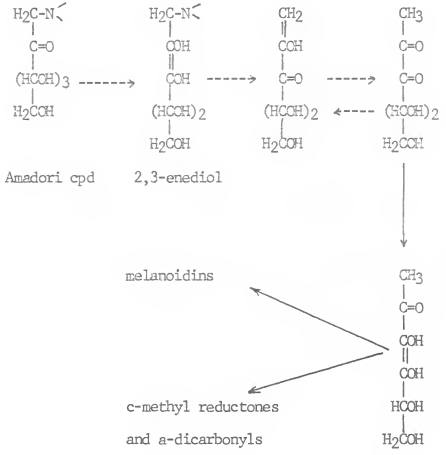

Figure 7. Minor Pathway for Carbonyl-Amine Reactions

Active-Aldehyde Theory

When the nonenzymatic browning reactions occur in the absence of nitrogenous compounds, they are described as caramelization reactions. Under anhydrous conditions upon the application of heat, or at high concentration in dilute acid solutions, the initial stages of the caramelization reaction are characterized by the formation of anhydro sugars. Glucose has been reported to yield glucosan and levoglucosan. The two anhydroglucose compounds are readly

distinguished by their specific rotation (Shallenberger and Birch, 1975). When sucrose is heated at about 200°C, simultaneous hydrolysis and dehydration occur, apparently followed by rapid dimerization of the products, so that a series of compounds characterized by isosacchrosan (corresponding to sucrose minus one molecule of water) is formed (Hodge, 1976). Isosacchrosan has no sweetness, but does have a mildly bitter taste.

In dilute solutions of reducing sugars, the initial stages of the caramelization, reactions are a series of events involving enolization, dehydration and fragmentation reactions. Subsequently, polymerization reactions occur, which lead to the formation of pigments similar to those formed in caramelization reactions at either higher temperature or in more concentrated solution.

The classic caramelizatin reaction is the phenomenon exibited by sucrose when subjected to heat, and the fundamental reactions are

- Inversion of sucrose to D-glucose and D-fructose
- Equilibration of anomeric and ring forms
- Condensation, intermolecular
- Condensation, intramolecular
- Isomerization of aldoses to ketoses
- Fragmentation reactions
- Browning

The caramelization reaction is autocatalytic and increasing temperatures not only increases the reaction rate but also alters the qualitative nature of the pigments (Doss and Ghosh, 1949).

The effect of pH is dramatic and the reaction rate at pH 8 is ten times greater than that at pH 5.9 (Ledon and Lananeta, 1950).

Caramelization reactions can be used either for coloring purpose, or for flavoring purpose. For flavor, sucrose is caramelized in concentrated syrups. The sugar fragmentation reactions are promoted by alkaline or neutral medium, whereby formation of humic substances is limited to avoid bitter, astringent tastes. Caramel for coloring use is produced in acid medium. Glucose syrup is treated with dilute sulfuric acid, partially neutralized with ammonia, then heated in the presence of a sulfite at a pH value of about 4 (Hodge and Osman, 1976).

Ascorbic Acid Theory

The exact route of ascorbic acid degradation is highly variable and dependent upon the particular system (Tannenbaum, 1976). Three different mechanisms of degradation of ascorbic acid have been proposed

- Catalyzed aerobic degradation
- Uncatalyzed aerobic degradation
- Anaerobic degradation

When oxygen is present in the system, ascorbic acid is degraded primarily via its monoanion (HA^-) to dehydroascorbic acid (DHAA). The exact pathway and overall rate is a function of the concentration of metal catalysts (M^{n+}) in the system. In the absence of oxygen the degradation pathway has not been yet verified. Following the suggestion of Kurata and Sakurai (1967), ascorbic acid is shown to react via its keto tautomer, H_2A-keto. The tautomer is in equilibrium with its anion, HA^- keto, which undergoes delactonization to diketogulonic acid (DKGA), as shown in Figure 8. Further degradation

beyond DKGA is closely related to nonenzymatic browning in some food products. The mechanism of these reactions has been studied by Kurata and Skurai (1967) under oxidative and non-oxidative conditions. One of the characteristic differences between the two pathways is that furfural is much more easily produced through the non-oxidative reaction. Under non-oxidative conditions, ascorbic acid in an acid solution was degraded to furfural with the formation of 3-deoxy-L-pentosone as an intermediate. This acid-catalyzed degradation took place under the storage or the cooking conditions of foodstuffs. It was shown that aldopentoses and 2-keto-L-gulonic acid themselves were not intermediates of the reaction. On the basis of their data, they assumed that the first step of the non-oxidative degradation of ascorbic acid in an acid condition is hydrolysis of the lactone ring followed by decarboxylation and dehydrations forming 3-deoxy-L-pentosone and furfural.

Factors Affecting the Browning of Packaged Orange Juice

Role of Ascorbic Acid

The primary oxidation product of L-ascorbic acid (AA) is dehydro-L-ascorbic acid (DHAA). The latter is not a stable compound; it undergoes spontaneous hydrolysis to a second product which has been well characterized as 2,3-diketo-L-gulonic acid (DKGA), formed by the opening of the lactone ring of dehydroascorbic acid. The first stage of oxidation to DHAA is reversible and the biological activity is retained; however, the oxidation of DHAA to DKGA is not reversible and the biological activity is lost.

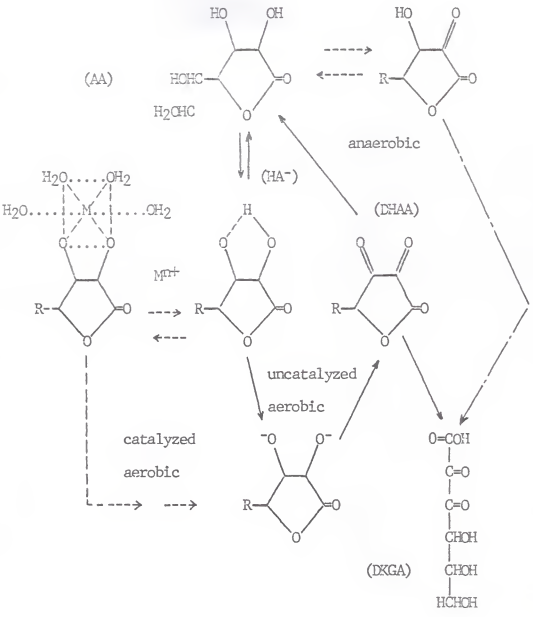

Figure 8. Initial Stage for Degradation of Ascorbic Acid (From Tannenbaum, 1976)

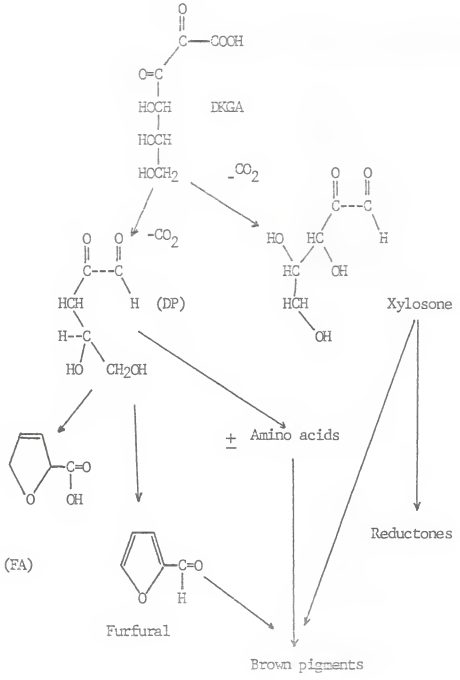

Figure 9. Degradation Pathway for Diketogulonic Acid (From Tannenbaum, 1976)

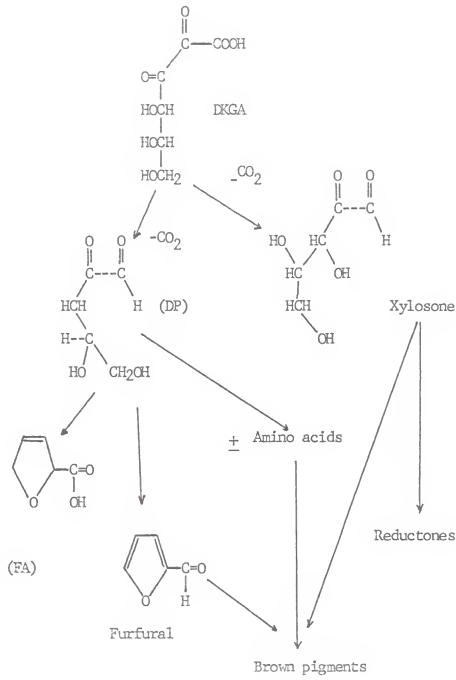

Figure 9. Degradation Pathway for Diketogulonic Acid (From Tannenbaum, 1976)

Further degradation of DKGA yields active products (such as furfural) which undergo polymerization, or react with nitrogenous constituents yielding brown pigments (Figure 9). These reactions may occur under aerobic as well as anaerobic conditions. For many years a relationship between browning in orange juice and AA loss has been known (Lamden and Harrisl, 1950; Joslyn and Marsh, 1935; Joslyn et al., 1934; Loeffer, 1941; Moore et al., 1942; Stephens et al., 1942; Joslyn, 1941; Curl et al., 1946; Curl, 1948; Clegg and Morton, 1965; and McWeeny and Burton, 1963). However, the exact role of AA in the discoloration of fruit products is still not well understood. It has been shown that when a citrus juice darkens carbon dioxide is evolved and AA is lost (Hamburger and Joslyn, 1941; Joslyn, 1957; Joslyn et al., 1934; Wilson, 1928; and Curl et al., 1946). This observed loss in AA during browning has led to the suggestion that AA is involved in the browning reaction in one of two ways: (a) It may serve as an antioxidant, being oxidized in preference to other substances present which upon oxidation yield compounds or precursors of dark compounds. (b) It may be oxidized along with other reducing constituents, such as the flavonols, as suggested by Szent Gyorgyi (1937), to yield different compounds that are the actual precursors of dark pigments.

Moore et al. (1942) showed that addition of AA to orange juice resulted in a marked increase in the rate of browning when juice is stored in the presence of oxygen. Similar results were obtained by Beattie et al. (1943) when they added AA (50mg/100ml) to strawberry juice. These data show convincing evidence that AA is involved in the browning of orange juice, and it is effective not as an antioxidant as

reported by Hamburger and Joslyn (1941), but rather as an intermediate in the browning reaction; otherwise, the addition of AA should retard rather than accelerate browning.

Wilson (1928) reported accumulation of carbon dioxide during the storage of sterile orange concentrate, and suggested that it may arise from the decomposition of AA. However, Eddy (1936) detected no carbon dioxide formation in his experiments on the oxygen absorption of unprocessed orange juice and AA solutions. Curl (1947), working on storage of pasteurized, concentrated orange juice at 26.7°C and above, noticed the formation of gas, which was shown to be essentially carbon dioxide. This gas does not result from fermentation. A number of possible sources of the carbon dioxide have been suggested, including the Maillard reaction between amino acids and sugars, decomposition or oxidation of AA, and a chemical breakdown of sugars. Later Curl and Veldhuis (1948) reviewed this subject and presented data showing that the formation of gas is accompanied by almost total losses of AA, significant losses of total sugars, and considerable darkening. Loeffer (1941) found that the quantity of carbon dioxide produced in canned orange juice during five month's storage at 35°C was almost equivalent to the quantity of AA lost. Lahikainen et al. (1958) showed, in model systems containing AA, glycine, and citrate buffers, and at concentrations corresponding to those found in fruit juices, that the carbon dioxide evolved during browning was not produced by decarboxylation of the glycine. This finding indicated that glycine had not undergone a Strecker degradation, although AA has been regarded as an active agent (Schonberg and Moubaker, 1952).

Lahikainen et al. (1958) reported that the rate of production of carbon dioxide was linearly related to the rate of browning at 37°C, but not at 50°C. This is in agreement with the finding of Joslyn, (1957) that a change in reaction mechanism may occur in AA systems between 30 and 50°C, and was later confirmed by Nagy and Smoot (1977) and Kanner et al. (1982). It is possible that pigment may be formed at higher temperatures by a separate mechanism or course of reaction than at the lower temperatures, or a second pigment producing reaction may be activated at the higher temperature. The total carbon dioxide produced was in excess of that required by the mono-decarboxylation of ascorbic acid.

Earlier, Landen and Harris (1950) found that in solutions of AA and citric acid there was no loss or breakdown of citric acid during the browning of the mixture when heated in closed tubes at 100°C. They found, also, that the quantity of carbon dioxide evolved from a 2.5% AA, 50% citric acid solution in five hour at 60°C was only 13.5% of the destroyed ascorbic acid. Jackson et al. (1960), using AA in acetate buffer, showed less than 4% of evolved carbon dioxide was derived from the acetate buffer. These findings indicated that evolution of carbon dioxide was due primarily to the multiple decarboxylation of ascorbic acid.

Joslyn (1957) reviewed earlier work on browning in model systems containing AA, and on the part played by AA in browning of orange juice. He reported that AA was the most reactive component in browning in systems containing AA, amino acids, and sugars. He observed that the rate of browning in orange juice was significantly

reduced when the anionic constituents were removed. However, the removal of the cationic constituents had less effect.

The rate of AA destruction in citrus products depends on several factors, including oxygen, storage temperature, pH value, metal catalyst and concentration of ascorbic acid itself.

Effect of oxygen. It is a generally accepted fact that oxygen has a pronounced effect on the rate of AA degradation and browning in orange juice. The processing operations involved in the citrus industry, such as extraction, screening, mixing and blending, increase the dissolved oxygen concentration of the products. The presence of oxygen is known to accelerate the AA destruction and the browning reactions. However, it has also been reported that the removal of molecular oxygen did not prevent loss of AA and darkening completly; and it has been shown that the AA degradation can proceed either aerobically or anaerobically. (Boyed and Peterson, 1945; Kefford et al., 1959; Nagy and Smoot, 1977; Nagy, 1980). One of the characteristic differences between these two reactions is that the anaerobic destruction is generally believed to proceed at a slower rate, with much easier production of furfural. An excellent review of both types of degradation has been published by Reynolds (1965). In early studies by Gore (1915) and McDermott (1916) it was observed that the color of citrus juices was more stable in the absence of oxygen. Matthews (1928) noted that samples of orange juice stored in air darkened more rapidly than those stored in oxygen-free gas atmospheres. Clark (1941) discussed the effect of an excess of oxygen in the sealed containers on color and flavor changes and reduction in

AA with possible darkening of the juice. Moore et al. (1942) were able to show that the rate of browning in pasteurized bottled orange juice was correlated with the volume of air-filled head space; as the volume of air was increased the rate of browning was also increased. Joslyn and Marsh (1932, 1934, 1935) and Joslyn et al. (1934) followed changes in vitamin C and browning in orange juice exposed to air. They reported that the browning of orange juice involves oxidation, and showed a decrease in vitamin C with an increased exposure to oxygen at room temperature. Browning was parallel to the loss of vitamin C, suggesting a possible relationship between the two processes.

Tressler et al. (1939) further commented that darkening was more rapid in the presence of oxygen. It also proceeded rapidly even when juice was deaerated to remove dissolved oxygen, and stored in vacuum sealed containers. Joslyn and Marsh (1935) concluded that the primary products of oxidation apparently undergo a condensation in which secondary reactions, probably of the amino acid sugar type, occur. In this connection, Joslyn (1941) suggested that the original, almost momentary, contact of orange juice with air before dearation may result in the formation of compounds (possibly peroxides) which supply oxygen for the later deterioration of juice even in the absence of oxygen. A number of papers have since appeared discussing the relationship of oxygen to quality in orange juice. Johnson and Toledo (1975) found that aseptically packaged 55° Brix orange concentrate lost 68% of its ascorbic acid content and was unacceptable after one week if oxygen was left in the package headspace. Completely

eliminating oxygen by storing the orange concentrate in glass containers with zero headspace considerably reduced AA degradation, and greatly extended the shelf life, but browning and flavor degradation still rendered the product unacceptable after ten weeks at 24°C. This does not exclude the possibility that the original, almost momentary contact of orange juice with air before dearation may form peroxides which supply oxygen for the later deterioration of the juice as previously suggested by Joslyn (1941).

The non-oxidative decomposition was studied at 30°C, and 100°C, from pH 2.2 to 6.0 (Huelin, 1953). In citrate-phosphate buffer the reaction proceeded most rapidly at pH 3-4, and was accelerated by fructose. Furfural and carbon dioxide were the main products of decomposition at high temperatures or acidities. With lowering of temperature or acidity other products became important. Cier et al. (1959) studied the anaerobic decomposition in an unbuffered solution of pH 2-6.6, and found L-xylose as well as carbon dioxide and furfural. However, xylose gave only traces of furfural at this pH, and did not appear to be the major intermediate between AA and furfural. Kurata and Sakurai (1967) studied the decomposition of AA at pH 2.2 and found 3-deoxy-L-pentosone and furfural, claiming the former as an intermediate. Coggiola (1963) also found acid products, and identified the major acid as 2,5 dihydro-2-furoic acid. Tatum et al. (1969), who used an unbuffered solution of AA and did not exlude oxygen, obtained 15 compounds as degradation products from AA, of which only furfural and 2,5-dihydro 2-furoic acid had been previously shown to come from non-oxidative decomposition of AA (Kamiya, 1959).

They suggested the condensation of furfural probably to be responsible for the formation of the two of the major components.

The kinetics of the oxidation reaction of AA have been reported by many researchers. Joslyn and Miller (1949a,b) reported the oxidation of AA in sugar solutions to be essentially first order with respect to the AA oxidation. Under conditions of limited oxygen, the sugar solution showed reduced initial rates of oxidation. Khan and Martel (1967) reported that the rate of the spontaneous oxidation of AA in an aqueous model system was proportional to the concentration of molecular oxygen down to 0.2 atm of oxygen. They also reported that the rates of ferric and cupric ion catalyzed AA oxidation were first-order with respect to the molecular oxygen concentration. Singh et al. (1976), working with infant formula, concluded that when dissolved oxygen is present in abundant supply, the reaction can be considered to follow a first order kinetics. However, with limited oxygen, (0.1-8.7ppm) the reaction followed second order kinetics. Eison-Perchonok and Downes (1982) studied the autoxidation of AA by varying temperature and AA and dissolved oxygen concentration. They reported that AA autoxidation is dependant on the dissolved oxygen concentration. However, Robertson and Samaniego (1986) observed no significant effects on the rate of asorbic acid degradation and furfural formation that could be attributed to the different initial oxygen levels in lemon juices. In summary one can say that there is considerable evidence that one important consequence of exposure to oxygen is the oxidation of AA which in turn is in some way responsible for browning of orange juice.

Effect of temperature. One of the most important factors influencing the rate of AA degradation is temperature. Many studies (Brenner et al., 1948; Freed et al., 1949; Bissett and Berry, 1975; Nagy and Smoot, 1977) have shown losses of ascorbic acid to be related to storage temperatures. VonLoesecke et al. (1934) reported that packs of orange juice in glass darkened if stored at temperatures of 27°C or higher, but not at 16°C or below. They further reported that the flavor of the juices in bottles and citrus-enameled cans was superior to that packed in plain tin cans. Pederson et al. (1941) observed a relationship of the temperature of storage to changes in flavor, color, and AA content. Loeffler (1941) stated that less than two months' storage at hot summer temperatures could make unpalatable the best quality of glass-packed orange juice, but that the flavor of the freshly bottled juice could be retained almost indefinitely at storage conditions around 4°C. He further remarked that the changes in bottled orange juice under warm storage temperature (35°C) were of the same order as those found by Vonloeseke et al. (1934) for samples packed in enameled-tin cans. Stephens et al. (1942) found the effect of storage temperature on the rate of darkening and stability of flavor to be roughly proportional to its effect on AA stability. This observation was later confirmed by Curl et al. (1946) who observed a parallel effect with respect to carbon dioxide production and thereby contributed support to the suggestion that carbon dioxide formation in these products is derived from a breakdown of AA (Joslyn et al., 1934; Nelson and Motter, 1933). Curl (1947), working with concentrated

orange juice, showed that the changes occurring at 27° and 49°C were similar, but the rate of change was about 20 times as fast at the higher temperature.

Brenner et al. (1948) and Freed et al. (1949) studied the retention of vitamin C in canned single strength orange juice at 21.1°, 32.2° and 37.8°C. These workers concluded that the logarithm of vitamin C retention was inversely related to storage time at these three temperatures. No statistical treatment was applied to their data to confirm their interpretation. Other investigators (Evenden and March, 1948; Joslyn and Miller, 1949b; Huelin, 1953) assumed that vitamin C degradation in orange juice was a first order reaction with the rate of degradation proportional to concentration. These findings differ significantly from those reported by Nagy and Smoot (1977) who found a nonlinear relationship between log percent vitamin C retention and time at high temperature. Their Arrhenius plot showed two distinct temperature regions. They defined a critical region between 22° and 26.7°C above which storage of juices resulted in an accelerated rate of vitamin C breakdown. For grapefruit juice the activation energy (Ea) was 18.2 kcal/mole, and the reaction was first order. For orange juice, two (Eas) were determined 12.8 kcal/mole in the temperature range 4-28°C, and 24.5 kcal/mole in the range 28-50°C. The change in reaction kinetics was attributed to different destruction mechanisms, although no explanation was offered. Kanner et al. (1982), studying the storage stability of orange juice concentrate packaged aseptically, showed that degradation of ascorbic acid follows first order reaction kinetics at temperatures of 25°C and

below. At 36°C the degradation of ascorbic acid did not follow a first order reaction. These data are in good agreement with the results of Nagy and Smoot (1977) on AA degradation in stored canned single strength orange juice, but differ from those of others (Brenner et al.,1948; Huelin, 1953), who found a first order reaction of AA degradation until 40°C or higher temperatures. Apparently, results differ because of the long storage time. During this period many breakdown products develop from juice constituents, which seem to affect and accelerate the degradation of AA (Clegg, 1964; 1966).

Role of metal catalysts. Metal ions can affect the deterioration of citrus fruits in two different ways: (a) metal ions may affect the browning pigment formation. Joslyn and March (1935) studied the effect of metal catalysts on the browning of orange juice, and reported that ferrous ions increased browning, stannous ions decreased it, while other metallic salts (including ferric, stannic, and copper salts) had no effect. Curl (1948) has shown that browning of a sugar-ascorbic acid system was increased by the presence of trace elements. Jackson et al. (1960) concluded that the addition of iron or copper decreased the rate of browning of aerated AA solutions buffered at pH 7, although the rate of loss of AA was increased. In the absence of heavy metals, DHAA and DKGA browned at a slightly faster rate than AA. (b) Metal ions may affect the ascorbic acid degradation. The catalytic properties of heavy metals on the oxidation of AA, notably copper and iron have been extensively discussed in the literature. Weissberger and LuValle (1944) reported that only the monoanionic AA species was susceptible to copper

catalysis. Later, studies by Khan and Martell (1967) showed the oxidation of AA in solution to be linearly dependant on the concentration of copper and iron ions. Shtamn and Shurlator (1974), and Jameson and Blackburn (1975) have also reported the catlytic properties of copper ions in the degradation of AA in solution. More recently, Dennison and Kirk (1982) studied the influence of trace mineral fortification on the storage stability of AA in a low moisture model food system as a function of water activity at a constant storage temperature of 30°C. They reported that AA degradation increased with increasing copper and iron levels. This concentration dependance is in accord with the results of Khan and Martel (1967) and Ogata et al. (1968).

It is somehow surprising that metal ions such as copper decrease browning, and at the same time increase the conversion of the AA present to DHAA and DKGA. This negative effect of copper placed some doubt on the correlation of the darkening of orange juice with loss of AA. Jackson et al. (1960) reported that metal ions in the presence of oxygen increase the conversion of AA to DHAA and to DKGA. If the formation of these compounds were the only limiting reactions in the browning of AA, their accelerated formation by metallic ions would indeed accelerate the rate of browning. Such is not the case, however, and in fact their results showed a negative catalysis. Conversely, the rate of browning in AA buffer systems devoid of metallic ions is increased when the available AA is converted to DHAA. These results would indicate that in a non-metallic system one of the first reactions in the presence of oxygen is a conversion of

the AA to its dehydro form and this conversion step may be partially rate-limiting. In the presence of metal, however, a reaction leading to the formation of polymeric compounds must be sensitive to metallic ions and becomes the rate-limiting reaction. It is possible that some intermediates beyond DKGA would chelate metallic ions, and if this were the case, the ability of these materials to form long chains capable of light absorption would be deleteriously affected, Hodges (1953).

The mechanism of metal catalyzed oxidation of AA in aqueous solutions is varied. Khan and Martell (1967) postulated an ascorbate-metal-oxygen complex involving a one electron transfer to oxygen. Jameson and Blackburn (1975) proposed the formation of a metal-metal dinuclear ascorbate-oxygen intermediate with a two electron transfer to oxygen. However, the exact mechanism of action of metal ions on the AA oxidation is still uncertain.

Effect of water activity. The nonenzymatic browning in foodstuff takes place over a wide range of water activity. In most foods a maximum browning reaction occurs at a certain value of a_w depending on the type of food product. It is generally agreed that the rate of browning in fruit juices and concentrates is increased as the water content in the product decreases (concentration of solids is increased), (Reynolds, 1969). Thus, nonenzymatic browning should occur more rapidly in dehydrated orange juice (1-3% water) than in frozen concentrated orange juice (55% water) or in single strength orange juice (85% water). It may be that the detrimental effect of

moisture on browning, so often observed, is actually due to an increase in the rate of AA destruction by oxygen uptake.

Karel and Nickerson (1964), Jensen (1967), Vojnovich and Pfeifer (1970) and Lee and Labuza (1975) have studied the stability of AA in various low and intermediate moisture dehydrated foods and model systems as a function of moisture content and water activity. Results reported by these investigators showed that the rate of destruction of AA in dehydrated foods increased as the total moisture content and a_w increased.

Kinetic data generated by Jensen, (1967), Vojnovich and Pfeifer (1970) and reported by Lee and Labuza (1975) have shown the energy of activation required for the destruction of AA to increase with moisture content in some foods but the opposite effect occured in the other foods. This could mean that a change in mechanism was occuring, but only limited data were collected. Lee and Labuza (1975) attributed the increased destruction rate for AA as a function of water activity to the decreased viscosity of the aqueous phase, resulting in increased mobility of reactant and catalysts.

Kirk et al. (1977) further noted that the destruction of AA could be described by first order kinetics under all storage conditions. The greatest stability of total ascorbic acid was observed at low storage temperature and water activity, and was shown to result from DHAA stability at these conditions. Due to availability of water for hydrolysis of DHAA, the stability of DHAA decreased at storage temperatures above 20°C and water activities above monolayer 0.24.

As previously mentioned, there is a certain value of a_w at which a maximum browning reaction occurs. By maintaining a_w level, either above or below the point of maximum browning, some increase in storage life could be obtained. Unfortunatly there is very little experimental evidence in the literature to define the range of a_w at which this maximum occurs in orange juice.

Effect of pH. It has been shown that the rates of browning in acidic foods such as citrus juices and in model systems are strongly pH dependent. Berry et al. (1970) studied the storage stability of dehydrated orange juice at pH values that ranged from 3.3 to 6.5 and found storage stability to decrease with increasing pH. They concluded that the greater storage stability of instant grapefruit juice (pH 3.3) over that of instant orange juice (pH 3.7) may be due to the greater acidity of the former.

Wolfrom et al. (1974) found the rate of browning in 1:1 glucose:glycine model system to increase as the pH was increased from 6.0 to 7.5, and the rate of browning in that pH range to be much greater than that between pH 3 and 4, the normal range for orange juice.

Braverman (1963) reported that the AA-induced browning in citrus juices and concentrates which initially involves its decomposition to furfural and subsequent polymerization reaction with amino compounds is dependent on pH, and within the pH range of 2.0 to 3.5 the extent of browning is inversely proportional to pH.

Horton and Dickman (1977) reported that AA is much less stable in phosphate buffer than in orange juice at the same pH. They suggested that factors other than pH must protect AA in orange juice against oxidation. Possibly citrate, a known chelator of heavy metal ions, inhibits the catalytic oxidation of AA. At higher pH values and at room temperature, all three reactions, (a) oxidation of AA to DHAA, (b) hydrolysis of DHAA to DKGA, and (c) the decarboxylation and further degradation of DKGA to a mixture of products, proceed much more rapidly in phosphate buffer.

Role of Nitrogenous Compounds

The nitrogenous constituents of citrus juices have long been suspected of being involved in the nonenzymatic browning of orange juice. Hall (1927) and Wilson (1928) were probably the first to suggest the possibility that the Maillard reaction was responsible for the darkening in citrus products. According to Hall it was definitely established that the amino nitrogen content of orange concentrates steadily decreases in storage and may drop to zero. Wilson found a reduction in amino nitrogen and reducing sugars, and suggested that darkening may be due to a Maillard reaction between sugars and amino acids. However, Nelson and Motter (1933) could detect no changes in the concentration of the nitrogen bases present in orange juice, and thus had reason to doubt Wilson's theory.

Joslyn and Marsh (1935) followed changes in amino nitrogen by means of the formol titration and also by the Van Slyke method. They observed that the amino-nitrogen level remained practically constant during the course of browning of Valencia and Navel juices, even after

storage for 126 days at room temperature when the juice had become very dark brown in color. They postulated the possible hydrolysis of soluble polypeptides during storage to account for the maintainance of constant amino nitrogen content during browning. The isolation of an alkali soluble protein from the chromatophores of the orange by Smith (1925), and later by Sinclair et al. (1935), lent support to this hypothesis. However, Stadtman (1948) doubted this explanation and stated that it would seem rather unlikely that the rate of amino nitrogen formation by such a process would exactly parallel the loss of amino acids through darkening. Nelson and Motter (1933) determined the distribution of nitrogen in various fractions of darkened and undarkened, filtered orange juice by the use of various precipitating agents. The darkened juice contained about twice as much soluble nitrogen as the fresh juice. No nitrogen constituents were found in the darkened juice which had not been identified in the fresh juice. There was, however, a considerable increase in the arginine content with darkening of the juice. Their results do not rule out the possibility that nitrogen compounds are involved in the browning of orange juice, but they do indicate that browning can occur without the transformation of large amounts of these substances. Loeffler (1941) also observed lower amino acid content in juices stored at 0°C than those stored at higher temperature.

The addition of certain amino acids to various fruit products has been studied. Thus, Matthews (1928) added asparagine in amounts varying from 0.01 to 0.1g/100 ml. to orange juice, with and without additions of 0.5g of glucose and citric acid. After a storage period

of one year, in air and nitrogen at 26.7-32.2°C, darkening in treated and untreated juice occured at the same rate and to the same extent.

Joslyn and March (1935) found that aniline, tryptophan, and other aromatic amines markedly increased the browning of orange juice. The effect varied greatly with the different compounds. Richert (1930) showed that free ammonia or ammonium ions greatly hastened the darkening of both sugar syrup and grape juice concentrates. Richert found glycine to be more effective than alanine but less so than ammonium tartrate. It is also common experience in the dried fruit industry that traces of ammonia, such as from leaks in refrigeration coils in cold storage, rapidly enhance darkening.

The confusion in the literature on changes in nitrogenous constituents during the browning of orange juice, may be due in part to failure to include the reaction of reducing sugars with proteins such as that found by Lea and Hannan (1950), and to the possibility that relatively small chemical changes are required to produce brown pigments of intense color. If this is the case, then the changes in reducing sugars, or amino nitrogen, necessary to produce large changes in color might be so small as not to be detectable by the methods ordinarily used.

Role of Sugars

The major sugars in citrus juices are sucrose, fructose, and glucose. Curl and Veldhuis (1947) reported the sugar composition of orange juice to be 5% sucrose, 2.5% fructose and 2.5% glucose. In order to see if reducing sugars were involved in browning of orange juice, Joslyn and Marsh (1935) studied the effect of their removal by

fermentation. The results showed that all samples (fermented and unfermented juice) darkened at about the same rate. However, how completly fermentation removed the sugars is not evident from the data. Stadtman et al. (1946) almost completely removed the reducing sugars from apricot syrups, yet the rate of browning was decreased to only about one half the rate in unfermented samples. The addition of fructose and glucose to fermented syrups in amounts equal to the sugar lost by fermentation, resulted in a restoration of the normal browning rate. These results indicate that sugar may not be involved in the darkening of orange juice, and that probably only part of the browning in apricots involves sugar reactions. Hall (1927), in a summary of work done on darkening in orange concentrates, stated that slight decreases in reducing sugar during storage have been observed. Curl et al., (1946) have verified this conclusion and showed that these losses in reducing value are roughly parallel to changes in color.

Following the suggestion that the Maillard reaction was responsible for browning (Wilson, 1928) a number of investigators have attempted to correlate browning with changes in reducing sugars. The initial rate of the Maillard reaction between a reducing sugar and an amino compound is directly related to the conformational stability of the favored cyclic structure of the sugar (Burton and McWeeny, 1963). Browning reaction in amino acid-sugar systems has also been shown to depend on the type of sugar (Cole, 1967; Spark, 1969) it follows that pentoses are more reactive than hexoses which are more reactive than disaccharides.

Wolfrom et al. (1974) studied the influence of different sugars on the browning reaction, and compared a 1:5 D-glucose-glycine system with similar systems in which D-glucose was replaced by D-fructose and sucrose. In the unbuffered system used, D-fructose showed a somewhat higher initial rate of browning than D-glucose. However, the authors stated that this difference might be reversed in the buffered media generally characteristic of foodstuffs.

Role of Container Type

Many studies have been made on the relationships between container, browning, and ascorbic acid retention in citrus products. Most early studies of single strength orange juice in metal containers or glass bottles generally showed that up to 75% or more AA was retained after one year storage at 26.7°C or lower (Moore et al., 1944). Single strength orange juice kept frozen in tin-lined cans at -17.8°C showed no change in AA concentration after one year (Nelson and Mottern, 1933).

In continuation of this work, Moore et al. (1944) compared the changes in color, flavor, and ascorbic acid content occurring during storage of the bottled and canned orange and grapefruit juices. At the end of six months' storage at room temperature, the orange juices in glass and tin were off-flavor, with the bottle juice slightly better in taste than the canned juice; the results would indicate that plain tin was found superior for packing orange juice with the exception that at room temperature the bottled orange juice retained a slightly better flavor during storage than the canned juice. Pasteurized single strength orange juice stored in glass bottles for 1

year retained 87% AA at 4.4°C but only 68% at 26.7°C (Curl and Veldhuis, 1947). AA retention in frozen concentrated orange juice in tin cans was 90% or greater after 1 year, at 4.4°C or below, regardless of headspace atmosphere, concentration of product, or preliminary heat treatments (Curl et al.,1946).

Curl (1947) also reported that loss of AA increased with temperature and concentration from 4.4 to 48.9°C and from 13 to 71° Brix, respectively. Retention at 4.4°C ranged from 99% for SSOJ to 93% for 71°Brix concentrate and at 26.7°C was reduced to 70% and 60%, respectively, after 1 year. DuBois and Kew, (1951) found frozen concentrated orange juice stored in tin-lined cans for 11 months at -28.9 to 23.9°C had very high (95% or more) retention of AA. Variation in frozen storage temperature had little effect on AA retention (McColloch et al., 1957) and samples stored from -12.2° to 15.6°C for 1 year, to simulate warehouse conditions, retained 95% of their AA.

Bissett et al. (1975) studied the AA retention in orange juice as related to container type. They found that single strength orange juice packed in glass retained about 90% of initial AA for over 4 months and 87% for 1 year at 4.4°C. AA retention was progressively less at 10 and 15.6°C (84 and 79% respectively).

PRELIMINARY STUDY

In a preliminary study, single strength orange juice was aseptically filled into pouches of 3 types of flexible film: retort pouch (American Can Company, Desmoines, Iowa.), vinylidene cryovac (W.R. Grace & Co., Cryovac Div., Duncan, SC.), and polyethylene Whirl-Pak (Nasco West, Modesto, CA.). These packaging materials posess different permeabilities to oxygen as indicated in Table 5.

Table 5

Permeability of the Packaging Film

Packaging film	Oxygen permeability cc O_2/100 sq.in./24hr. 72°F., 1mil.
Retort pouch	0[a]
Vinylidene cryovac	0.1-0.2[b]
Polyethylene Whirl-Pak	500[b]

[a]-Adams (1982)
[b]-Sacharow and Griffin (1970)

The pouches were overfilled and sealed through the juice to prevent the inclusion of air. The pouches were stored at room temperature (22 + 3oC) and analyzed at 0, 2, and 4 week intervals. Ascorbic acid and dehydroascorbic acid concentrations were determined using the automated fluorometric method of Roy et al., (1976). The Autoanalyzer technique called segmented flow analysis, where the sample and reagent streams are segmented by air bubbles, and continuously pumped with automated sampling of sample solutions was

used. The procedure is based on the oxidation of ascorbic acid to dehydroasccorbic acid by N-bromo succinimide followed by condensation of the dehydroascorbic acid with o-phenylenediamine to form a fluorophore. The fluorescence is then measured using a fluorometer with a flow-through sample cell. The dehydroascorbic acid can be determined by omitting the oxidation step of ascorbic acid.

This procedure was found accurate only when used with fresh samples of orange juice. The accuracy of the method was determined by comparing the analytical results obtained by the continuous flow procedure with the titration method with 2,6-dichlorophenolindophenol (AOAC, 1975). Table 5 shows that the two procedures were significantly different after 2 weeks storage. At this time some of the degradation products in the orange juice samples interfered with the fluorescence measurement. Therefore, an HPLC procedure was investigated which allowed the simultaneous measurement of ascorbic and dehydroascorbic acids in fresh samples as well as in browned samples of orange juice using a single injection analysis procedure.

Table 6

Comparison of the Fluorometric and the Dye Titration Procedures for Ascorbic Acid Determination

	0-WEEK		2-WEEK		4-WEEK	
	a	b	a	b	a	b
Retort pouch	26.5	27.7	21.4	26.7	18.6	26.0
Cryovac pack	26.7	27.0	10.0	17.4	8.3	16.9
Polyethylene	22.0	27.5	0.3	0.4	0.2	0.3

a = fluorometric procedure
b = dye titration procedure.

SIMULTANEOUS ANALYSIS OF ASCORBIC AND DEHYDROASCORBIC ACIDS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH POST COLUMN DERIVATIZATION AND UV ABSORBANCE

Introduction

Fruits and vegetables constitute the major sources of vitamin C for human diets. The total vitamin C consists of the sum of ascorbic acid and its oxidized form, dehydroascorbic acid. Both forms have equal antiscorbutic activity (Tannenbaum, 1974).

Numerous methods for the analysis of vitamin C activity have been described. The most commonly used are the 2,6 dichlorophenolindophenol visual titration (AOAC, 1975), the spectrophotometric method with dinitrophenylhydrazine derivatization of DHAA (Roe et al., 1948), and the microfluorometric method by condensation of DHAA with OPDA (AOAC. 1975). However, these methods are not specific and are often limited by the number of interfering substances present in foods. In addition, it is difficult to determine visually the end point when these methods are used with colored solutions. Pelletier and Brassard (1977) described an improved photometric method based on 2,4-dinitrophenylhydrazine for the AA and DHAA determination in foods. Though their method eliminated interference from other compounds, it was time consuming and requires special sample preparation.

Recently, due to the development of commercial HPLC systems, quantitative measurement of AA and DHAA in various substances has been reported by many investigators. Procedures vary in the type of

column, elution conditions, detection systems and the extraction technique used to stabilize AA and DHAA. AA can be determined easily by HPLC, with UV detection, but the determination of DHAA is complicated by its extremely low UV absorptivity. A procedure using two reversed-phase HPLC columns in series for the separation of AA and DHAA was reported by Finley and Duang (1981). They used water with a counter-ion reagent (tri-n-butylamine) as a mobile phase. AA and DHAA were detected at 254 nm and 210 nm respectively. Rose and Nahrwold (1981) and Wimalasiri and Wills (1983) used a similar detection system with a single ion-exchange column and a mobile phase of acetonitrile-water containing 2.5 mM potassium dihydrogen phosphate. Doner and Hicks (1981) reported a separation of AA and DHAA by HPLC on a Zorbax-NH_2 column. The AA was monitored at 268 nm, while refractive index (RI) detection allowed the detection of DHAA. However, neither RI nor low wavelength (210 nm) can detect small amounts of DHAA such as that present in foodstuffs. In addition, measurement of DHAA at low wavelengths introduces instrumental noise from solvent impurity.

Therfore, most of the HPLC analytical procedures used are based on either the reduction of DHAA to AA and detection of the total ascorbic acid (TAA) by UV or oxidation of AA to DHAA. The TAA is determined by fluorometry after condensation of DHAA with OPDA. Dennison et al. (1981) described an HPLC method for the analysis of total vitamin C in beverages by UV measurement of AA after reduction of DHAA with homocysteine. Keating and Haddad (1982) reported the simultaneous determination of AA and DHAA using precolumn derivatization. DHAA was converted to a fluorophore using OPDA.

The detection was made at 290 nm for AA and 348 nm for the fluorophore. Speek et al. (1984) developed an HPLC method for the simultaneous determination of total vitamin C based on precolumn enzymatic oxidation of AA to DHAA. The latter is condensed with OPDA and detected fluorometrically. DHAA can be determined with omission of the oxidation step.

While these methods give increased sensitivity for the estimation of DHAA, the addition of the derivatization step increases the complexity and adds another variable to the analysis. Also, problems were encountered with the stability of the derivative.

Recently, Vanderslice and Higgs (1984) proposed an HPLC method with fluorometric detection and post-column derivatization involving oxidation of AA to DHAA followed by reaction with OPDA to form a fluorescent product.

In this study we have examined the system proposed by Vanderslice and Higgs (1984) and modified it to obtain an estimation that includes AA and DHAA as a separate value using a single injection. The step for oxidation of ascorbic acid was also omitted.

Materials and Methods

Reagents

Ascorbic and dehydroascorbic acids (Aldrich Chemical Company, Inc. Milwaukee, Wis.), o-phenylenediamine (OPDA) (Eastman Kodak Company, Rochester, N.Y.), metaphosphoric acid, potassium phosphate monobasic, and HPLC grade acetonitrile (Fisher Scientific Company, Fair Lawn, N.J.) were used as received. Double distilled deionized water was used to prepare solutions.

Apparatus

Liquid chromatograph. High performance liquid chromatography was performed using a system incorporating a Waters Associates Model 6000A pump, a Waters Model U6K injector, a Spectra Physics, Model 8440 variable-wavelength ultraviolet detector (Spectra-Physics, San Jose, CA.) set at 254 nm, and a Fisher Recordall Series 5000 recorder. Separation of AA from DHAA was achieved by use of an amine column (Alltech Associates, Inc., Applied Science Labs, Deerfield, IL.) in the weak anion exchange mode. The mobile phase was 75% acetonitrile in 0.05M monobasic potassium phosphate (pH=5.9). The eluant was filtered through a 0.45 um Millipore filter (Gelman Sciences. Inc., Ann Arbor.Mich.), and subsequently degassed under vacuum. The flow rate was 1.5 ml/min.

Post-column Derivatization

The system used for the post-column derivatization was similar to that described by Vanderslice and Higgs (1984). After separation of AA and DHAA on the analytical column, the exit stream from the UV-detector was mixed with a second stream containing the derivatization reagent (OPDA) in a mixing "PTFE Tee" (Rainin, Woburn, MA., Catalog No. 45-1003). The final eluant was passed through a heating coil then into a cooling coil before entering a fluorometric detector (Aminco Fluoromonitor) equipped with an ultraviolet mercury light source (type GE no.F4T4/BL,4watt), a Corning 7-51 exitation filter, a Wratten 2A emission filter and a 70uL flow cell, whose output was sent to the recorder, Fig 10. All post-column tubing was 0.40 mm i.d. Teflon®. The reaction path length was 20 meters and was

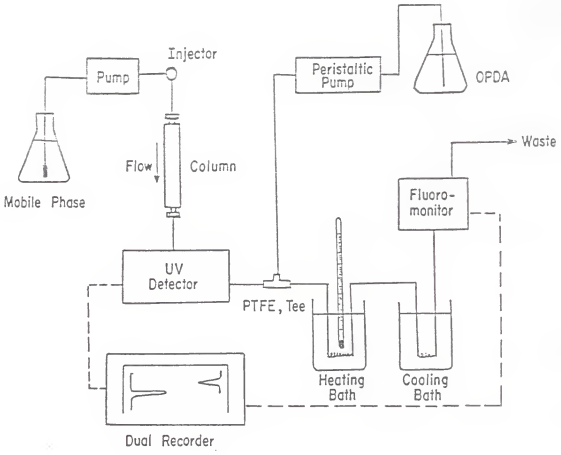

Figure 10. HPLC System with Post-Column Derivatization and Tandem Ultraviolet and Fluorometric Detection

maintained at constant temperature (63°C), while the cooling coil (22°C) was 2 meters. The fluorogenic reagent consisted of 0.05% (w/v) OPDA in distilled water and was pumped using a Gilson Minipuls 2 peristaltic pump at a flow rate of 0.5 mL/min.

Sample Preparation

Fresh fruits and vegetables were purchased from a local market and homogenized in a domestic blender. A sample (20g) was then blended with 3% (w/v) metaphosphoric acid solution (50mL) for 2 min and diluted to volume (100 or 200 ml) with extracting solution. The resulting solution was filtered through paper (Whatman 541), and a portion of the filtrate was purified by percolation through a C_{18} Sep-Pak (Waters Associates, Milford, MA), a short plastic column containing uBondapak C_{18} as described by Wimalasiri and Wills (1983). The C_{18} Sep-Pak was placed on the Luer tip of the syringe barrel and the column preconditioned with 4mL of methanol followed by 10mL of water. The sample (4mL) was then passed through the Sep-Pak. The first 3mL were discarded and the remaining 1mL was collected for analysis. The Sep-Pak C_{18} could be reused up to eight times provided it was washed with methanol and water between samples. Frozen orange juice was first diluted according to package directions. The resulting solution was filtered and purified as above. The injection volume was 20 uL.

Recovery Study

Proper amounts of ascorbic acid and dehydroascorbic acid standards were added as solutions in metaphosphoric acid to the various fruits and vegetables during extraction so that the AA and

DHAA content of the spiked samples approximately doubled that of the unspiked. The AA and DHAA of spiked samples were then determined as described previously and percent recovery was calculated.

Calibration Curves

Samples of reagent grade AA and DHAA in the mobile phase were combined to contain 2.0 AA + 0.5 DHAA, 4.0 AA + 1.0 DHAA, 6.0 AA + 2.0 DHAA, 8.0 AA + 3.0 DHAA and 10.0 AA + 4.0 DHAA mg/100ml. The standard mixtures had to be prepared daily. Aliquots (20ul) of the combined solutions were injected into the chromatographic system, and the resulting peak heights were plotted against concentrations for the calibration curve.

Results and Discussion

Using the HPLC procedure described, linear calibration curves were obtained for DHAA in the range 0-4mg/100ml and for AA in the range 0-10mg/100ml. Correlation coefficients of the linear regression equations were 0.9994 for AA and 0.9999 for DHAA, and the limits of detection were 0.05ug for AA and 0.01ug for DHAA per 20 ul injected. The signal to noise ratio was S/N = 8.

Typical chromatograms of orange juice, parsley, tomato, and strawberry are shown in Fig 11 and Fig 12. These illustrate the ability of tandem ultraviolet-fluorometry detection to determine simultaneously AA and DHAA. In all samples, the AA and DHAA peaks were well resolved with no interference. The DHAA peak had a shoulder in some of the samples. The procedure was successfully applied to the analysis of vitamin C in different food products and the results are presented in Table 6. Although direct comparison was not made with

other methods of analysis such as the dye titration or the fluorometric method, the levels of AA and DHAA were found to be similar to those reported in the literature (Ashoor et al., 1984; Wills et al., 1983; Wimalasiri and Wills, 1983).

Table 7

Ascorbic Acid and Dehydroascorbic Acid Content of Various Foods.

Sample[a]	Concentration (mg/100g)		
	AA	DHAA	TAA
Broccoli, fresh	81.5 ± 2.5	6.2 ± 0.9	87.7 ± 1.6
Orange juice	42.7 ± 1.1	2.9 ± 0.2	45.6 ± 1.0
Fruit punch	51.0 ± 1.9	3.9 ± 0.2	54.9 ± 2.1
Orange drink	45.7 ± 1.6	1.0 ± 0.1	46.7 ± 1.5
Parsley	148.7 ± 7.4	9.7 ± 1.0	158.5 ± 7.0
Tomato fresh	9.1 ± 0.4	1.1 ± 0.2	10.2 ± 0.5
Strawberry	51.0 ± 1.9	6.1 ± 0.2	57.1 ± 1.8
Banana	7.7 ± 0.6	3.0 ± 0.1	10.7 ± 0.6

[a] Number of samples = 4. Mean ± SD

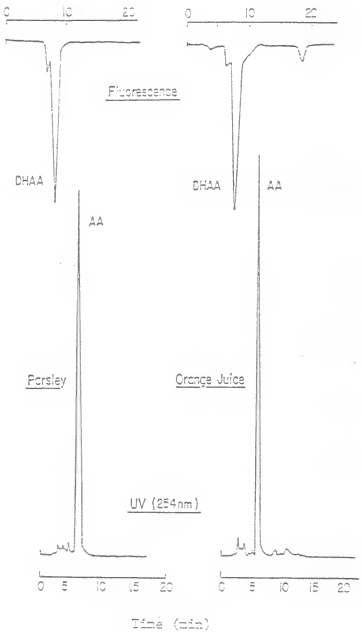

Figure 11. Typical HPLC Chromatograms of Orange Juice and Parsley, Monitored by Tandem Ultraviolet (UV, 254 nm) and Fluorometric Detection

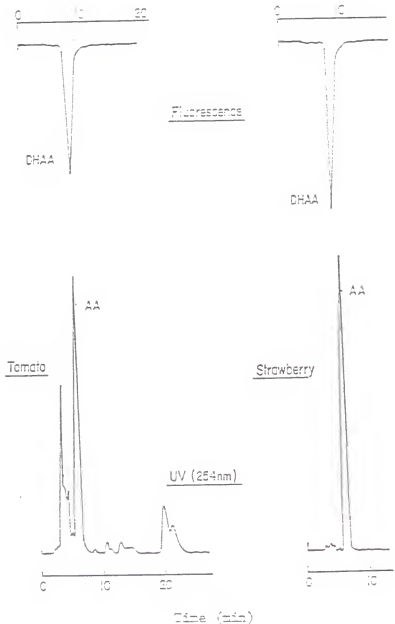

Figure 12. Typical HPLC Chromatograms of Tomato and Strawberry Monitored by Tandem Ultraviolet (UV, 254 nm) and Fluorometric Detection

Table 8

Recovery of Ascorbic and Dehydroascorbic Acids from Spiked Samples

Sample[a]	Recoveries (%)		
	AA	DHAA	TAA
Broccoli,	97.7 ± 2.9	110.7 ± 4.6	104.2 ± 3.5
Orange juice	99.5 ± 2.7	101.7 ± 0.8	100.6 ± 1.8
Fruit punch	97.0 ± 0.7	102.5 ± 1.1	99.8 ± 0.9
Orange drink	96.7 ± 1.5	101.7 ± 2.2	99.2 ± 1.8
Parsley	91.0 ± 1.9	111.7 ± 2.5	101.4 ± 2.2
Tomato	96.0 ± 1.2	104.7 ± 1.5	100.4 ± 1.3
Strawberry	96.5 ± 1.1	105.7 ± 1.5	101.0 ± 1.3
Banana	91.2 ± 1.9	102.5 ± 1.1	96.9 ± 1.5

[a] Number of samples = 4. Mean ± SD.

The data in Table 7. show that both AA and DHAA are completely recovered from the samples examined. The recoveries ranged from 91 to 99.5% for AA and from 101 to 112% for DHAA. The slightly higher recoveries for DHAA could be due to the oxidation of some of the AA to DHAA during extraction and sample preparation. For samples that require homogenization and extraction such as banana and parsley etc., conducting the extraction at 3°C should be helpful in preventing potential conversion of AA to DHAA during the extraction procedure.

This HPLC procedure provides a relatively fast and sensitive technique for the simultaneous determination of AA and DHAA in foodstuffs and beverages. The method is simple and requires a minimum of sample preparation during the simultaneous determination of AA and DHAA. Further, this HPLC method measured AA and DHAA directly, which

eliminates the need for the oxidation of AA to DHAA or the reduction of DHAA to AA prior to the analysis. The procedure was also found to be very useful for measurement of AA and DHAA in browned samples of orange juice where many interfering compounds limited the use of the dye titration method and the microfluorometric method.

Finally, an attempt was made to include the diketogulonic acid (DKGA) determination in our assay. The DKGA was prepared according to the method of Doner and Kevin (1981) as follow: DHAA was readily prepared from AA by air oxidation of an ethanolic solution containing activated charcoal. After filtration and removal of ethanol, pure syrup of DHAA was obtained, as determined by HPLC analysis. DHAA (an aqueous solution of 10mg/ml) was then converted to DKGA by gradual titration in an ice bath over a period of 1 hour with an aqueous solution of 10mg/ml with 0.5N sodium hydroxide until the pH remained constant at 7.0. Standard mixtures of AA, DHAA, and DKGA were prepared just prior to analysis by HPLC (UV detection). The acquisition of a new Hewlett Packard 1090 Liquid Chromatograph with HP-85B Personal Computer and DPU multichannel integrator, permitted the simultaneous monitoring of the different compounds at different wavelengths. Figure 13 presents a chromatogram of the standard mixture (AA, DHAA, and DKGA) with their absorption spectra. This procedure provided an excellent resolution of the three compounds with retention times of 7.9, 3.7, and 10.7 minutes respectively. However, the UV detection of DHAA and DKGA was not sufficiently sensitive to detect these two compounds at the levels actually present in orange juice, even when a low wavelength (210 nm) was used for analysis.

Figure 14 shows two chromatograms of AA (40 ug/ml) and DHAA (1 mg/ml) at two different wavelengths (210 and 254 nm). At the level used DHAA was only slightly detected at 210 nm. This demonstrates the advantage of the fluorometric detection procedure where the sensitivity was significantly improved over UV detection. Measurement of DHAA and DKGA has been made by others using UV detection (Finley and Duang, 1982). We found the maximum absorption for DHAA occurs at a wavelength of 227 nm, and for DKGA _ 200 nm, figure A-6. Many other compounds which occur in biological samples also absorb at these low wavelengths. Therefore, determination of DHAA and DKGA by UV detection was not a suitable procedure for the low levels in orange juice.

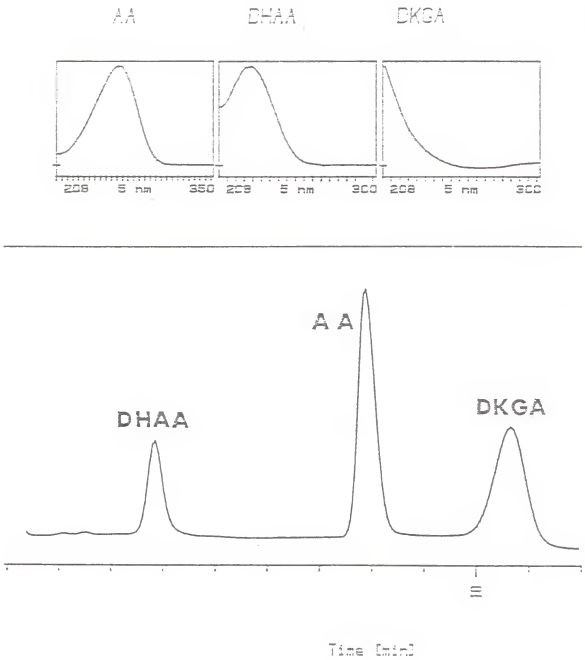

Figure 13. Resolution of AA, DHAA, and DKGA

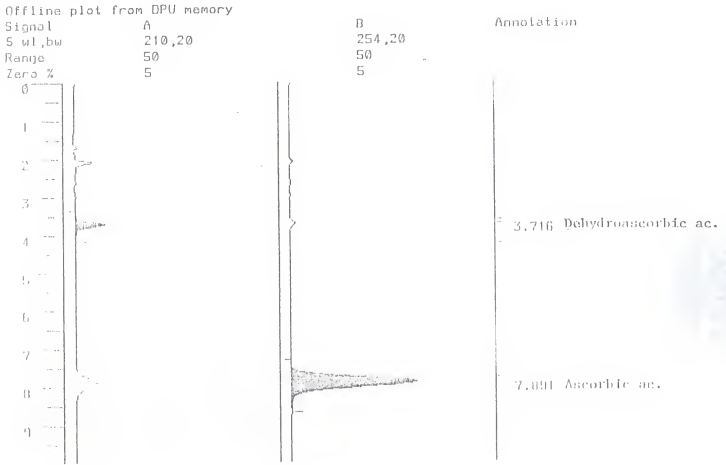

Figure 14. Ascorbic and Dehydroascorbic Acids Monitored at 210 and 254 nm

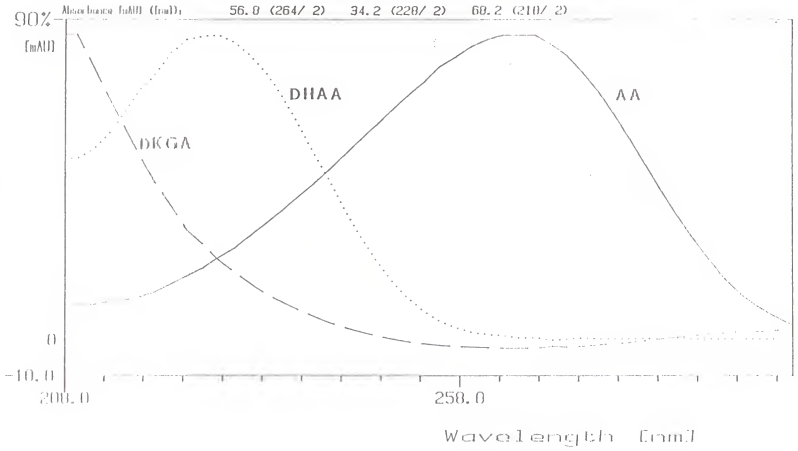

Figure 15. Absorption Spectrum for AA, DHAA, and DKGA

EFFECT OF ASCORBIC ACID AND AMINO ACIDS CONCENTRATIONS ON THE QUALITY OF ASEPTICALLY PACKAGED ORANGE DRINKS

Introduction

Nonenzymatic browning is one of the main reasons for the reduction in commercial value of citrus products. It is the quickest and most dramatic quality defect to appear during ambient temperature storage. Knowledge of the fundamental factors and the mechanism of reactions which these factors can undergo under different storage conditions is critical to the understanding and subsequent control of nonenzymatic browning in citrus. While some progress has been made in the study of the changes responsible for darkening, the chemistry of many of these changes is not well understood, and the nature of all the constituents involved is not known.

In view of this lack of understanding, it was decided to carry out the present investigation to study the reactions taking place during the nonenzymatic browning of aseptically packaged orange drinks. Our main objectives were to determine the loss of ascorbic acid and the development of browning as affected by ascorbic acid and amino acid content, and oxygen permeability of packaging material.

A 3x3x2 factorial experiment was designed to measure the main effect of ascorbic acid, amino acids, and oxygen permeability alone as well as all permutations of any two or three factors' interaction on the browning of orange drinks aseptically packaged and stored at 75°F.

Materials and Methods

Reagents

Ascorbic acid (Aldrich Chemical Company, Inc. Milwaukee, Wis.), arginine, aspartic acid, citric acid, potassium citrate, fructose, and glucose (Fisher Scientific Company, Fair Lawn, N.J.), 4-aminobutyric acid (Eastman Kodak Company, Rochester, N.Y.) and sucrose (local market) were obtained and used as purchased. The juice used in this study was reconstituted from a high quality Florida commercial concentrate. The frozen concentrated orange juice was first diluted according to package directions with distilled water which was boiled then cooled to room temperature to remove any dissolved oxygen. The final degree Brix was 11.8.

Orange Drinks Composition

Nine orange drink mixtures containing 10% (w/w) orange juice and various compositions of ascorbic acid, amino acids, sugars, ciric acid, and potassium citrate were prepared, aseptically packaged, and stored at controlled room temperature (75°F, 23.9°C). The compositions of the mixtures are given in Table 9. Analyses of the mixtures were conducted over a period of 20 weeks.

The mixture of sugars used (5% sucrose, 2.5% glucose, and 2.5% fructose) was similar to that which occurs in orange juice, (Curl and Veldhuis, 1947). For amino acids, a mixture of 0.2% each of L-aspartic acid, L-arginine, and 4-aminobutyric acid was used. Since these are the most abundant amino acids in orange juice and in many other fruit juices (Winston, 1961), it was considered that these amino acids may play an important role in the deterioration of orange juice

on storage. Wolfrom et al. (1974) reported L-arginine and 4-aminobutyric acid to give the most intense and rapid color formation, and were quantitatvely much more effective than glycine or any other of the 9 amino acids examined. According to the literature orange juice contains 0.6%(w/w) of crude proteins (Chatfield and Adams, 1940; and Watt and Merrill, 1963), hence this quantity of the mixed amino acids was used in mixtures, 2, 5, and 8. To mixture 1, 4, and 7 no amino acids were added, and the only amount present in the mixture is that contributed by the 10% orange juice. This amount was assumed to be 0.06%. To mixture 3, 6, and 9 amino acids were added to the level of 1.26% (w/w).

The quantity of ascorbic acid used in the orange drinks 4, 5, and 6 was 38.0 mg/ 100ml. To mixtures 1, 2, and 3, no ascorbic acid was added, and the mixture content was 4.2 mg/ 100ml, provided by the 10% orange juice. Mixture 7, 8, and 9 contained 71.8 mg/ 100ml of ascorbic acid. Each mixture contained 1% (w/w) of citric acid and 0.7% (w/w) of potassium citrate (both as hydrates) to buffer the solutions to a pH of about 3.8, which falls within the normal range of pH of orange juice. These compounds are part of the buffer system of orange juice. The mixtures were filled aseptically at room temperature.

With mixture 1, 4, and 7 the ascorbic acid level was increased (4.2, 38.0, and 71.8 mg/100 mL), the objective was to determine the effect of the concentration of ascorbic acid on the rate of ascorbic acid loss and the rate of browning without addition of amino acids. The amino acid level (0.06%) was that naturally provided by the 10%

Table 9

Composition of the Orange Drink Mixtures

Mixture	Ascorbic Acid (mg/100ml)	Amino acids[a] % (w/w)	Other constituents % (w/w)
M 1	4.2	0.06	All mixtures
M 2	4.2	0.66	contained
M 3	4.2	1.26	1 % citric acid 0.7 % potassium citrate
M 4	38.0	0.06	
M 5	38.0	0.66	5 % sucrose
M 6	38.0	1.26	2.5 % glucose 2.5 % fructose
M 7	71.8	0.06	
M 8	71.8	0.66	
M 9	71.8	1.26	
M 0	42.0	0.60	S.S.O.J.

[a] A mixture of equal amounts of aspartic acid, 4-aminobutyric acid, and arginine was used in each case with the indicated percentages.

orange juice. With mixture 2, 5, 8, and 3, 6, 9 the objective was to determine the effect of increased levels of amino acids (0.66 and 1.26%) on browning and ascorbic acid degradation.

Preparation of the Mixtures

Using the facilities at the Food Science and Human Nutrition Department (University of Florida, Gainesville), the different ingredients were mixed with single strength orange juice and distilled water to make an orange drink containing 10% orange juice, the final pH was 3.8. Three mixtures were prepared each day. The filling of

the pouches was done on the day following the preparation of the mixtures. The mixtures were maintained overnight at 4°C before processing and packaging.

The juice and the drinks were processed using a No-Bac Unitherm IV Processing system. (Cherry-Burrell Corporation, Cedar Rapids, Iowa). It is a complete unitized system for sterilizing fluid products at a rate of 22.5 to 45 gallons per hour. It consists of two surge tanks, one with agitator, supply pump, high pressure pump, heat exchangers, aseptic remote homogenizing valve, valve manifold and control panel. The high pressure pump has a 3000 PSI maximum pressure limitation. The 1/4" tubular heat exchangers have a 150 PSI maximum steam pressure limitation. The system was first sterilized with circulating water at 285°F for 20 minutes. The product was pumped from an agited supply tank and was heated to 205°F for 14 seconds, with the flow rate of the product kept at 31.5 gallons per hour. The sterile product was then immediately cooled to 80°F. From this point on every precaution was taken to make certain the product did not become recontaminated.

The products were then packaged into 7 by 10 cm pouches of two types of flexible films: retort pouch (zero permeability to oxygen) and polyethylene pack (high permeability to oxygen). The retort pouch composition from inside was polyethylene terephthalates/aluminum foil/polypropylene. They were steam sterilized for 8 hours. The polyethylene packs were Whirl-Pak type, commercially sterile, and were used without further treatment. The pouches were aseptically overfilled and sealed through the liquid to prevent the inclusion of

air. The filling was made in a sterile environment in a laminar flow hood (The Baker Co., Inc. Sanford Airport, Sanford, Me.). The hood was equipped with a blower that provided an average air velocity of 99.6 fpm., a prefilter-Scottfoam (washable), a final filter (Zero Probed HEPA, 99.99% efficient on all particules 0.3 micron by D.O.P. test) and an UV light to maintain the sterile conditions. Thirty two pouches were prepared from each mixture and each packaging material. Eight pouches from each mixture (4 retort pouches and 4 Whirl-Pak) were opened after each storage period and analyzed for ascorbic acid, dehydroascorbic acid, and browning over a period of 20 weeks.

Method of Analysis

Ascorbic and Dehydroascorbic Acids Determination

Ascorbic and dehydroascorbic acids were determined as soon as possible after the pouches were opened using the HPLC procedure previously described. The zero-time analysis was made on the day following the filling of the pouches. The pouches were allowed to stand overnight at room temperature. Thereafter, samples were analyzed at 2-week intervals for the first 8 weeks, and then at 12, 16 and 20 weeks. All measurements were made on 4 packages from each mixture.

Browning Measurement

Browning expressed as absorbance at 420 nm, was measured according to the method developed by Meydav et al., (1977). In this method the pulp and serum were separated centrifugally (2000 rpm for 20 min). The supernatant was then diluted 1:1 (v/v) with ethyl alcohol (95% v/v) to cause floculation of the cloud and was then

filtered through a Whatman no.42 filter paper to obtain a fully clarified extract. The clear solution was checked for its absorbance at 420 nm in an LKB 4050 Ultrospec. (LKB. Biochrom LTD Science Park Cambridge, England).

Statistical Treatments

Regression methods were used for the calculation of factor effects, and for the analysis of variance (ANOVA).

Results and Discussion

Ascorbic Acid Retention

Effect of processing. From a nutritional point of view, the retention of AA is an important factor for citrus products. Table 10 shows the estimated initial and zero time AA and DHAA levels for orange juice and orange drinks products in both types of packaging materials. There was considerable discrepancy of AA between the initial estimated values, and the measured value at zero time. This loss occurred in all mixtures and in both types of packaging materials, although it was more pronounced in the Whirl-Paks. This decrease in AA level represents the combined loss during processing, storage of the sample prior to the first analysis, and preparation of the drinks. The incorporation of oxygen into the mixtures prior to thermal processing (ie. dilution with oxygen containing water, mixing in open kettle to dissolve the ingredients) may have contributed to a large extent to the oxidation of AA to DHAA and the conversion of DHAA to DKGA. This is evidenced by the high level of DHAA found in all mixtures at the zero time. On average the percent loss for total ascorbic acid (TAA) ranged from 12 to 21% in retort pouches and 20 to 26% in the Whirl-Pak.

Table 10

Initial AA and DHAA Levels for Orange Juice and Orange Drink Products in Retort Pouch and Polyethylene Pouch

	Initial estimated values (mg/100 mL)			Zero time (mg/100 mL)					
				Retort pouch			Polyethylene pouch		
Pdt	AA	DHAA	TAA	AA	DHAA	TAA	AA	DHAA	TAA
M0	42.4	3.0	45.4	31.5±.6	4.1±.1	35.6	30.6±.6	5.3± .5	35.9
M1	4.2	0.3	4.5	1.0±.2	2.3±.2	3.3	.3± .0	2.4± .2	2.7
M2	4.2	0.3	4.5	.9±.3	2.4±.2	3.3	1.1± .1	2.1± .1	3.2
M3	4.2	0.3	4.5	1.4±.1	2.3±.1	3.7	1.3± .2	2.3± .1	3.5
M4	38.0	0.3	38.3	19.6±.3	13.6±.4	33.2	12.2± .9	15.4± .9	27.6
M5	38.0	0.3	38.3	20.1±.8	11.2±.4	31.3	15.9± .4	13.0± .8	28.9
M6	38.0	0.3	38.3	16.1±.9	11.9±.4	28.0	13.5± .5	14.1± .4	27.6
M7	71.8	0.3	72.1	51.6±.7	12.5±.1	64.1	44.0±1.7	16.5±1.1	60.5
M8	71.8	0.3	72.1	50.6±.9	13.8±.9	63.8	32.2±2.4	24.1±1.9	56.3
M9	71.8	0.3	72.1	46.0±.7	15.6±.7	61.6	35.8±1.2	19.5±1.0	55.2

[a]Number of samples = 4. Mean ± SD.

Ascorbic acid retention as affected by amino acid concentration.

The effect of amino acid level on ascorbic acid retention and the concentration of its oxydation product, dehydroascorbic acid, are presented in Figures 16 to 19. Figure 16 shows the loss of ascorbic acid in samples stored in Whirl-Paks initially fortified with the highest concentration of ascorbic acid (71.8 mg/100 ml) and increasing levels of amino acids. In addition to the initial rapid loss of AA, these samples continued to lose AA more rapidly in storage than did those in retort pouches and by the second week of storage both AA and DHAA had almost completly disappeared. Similar results were obtained at the 38.0 and 4.2 mg/100 ml ascorbic acid levels (data in Appendix Figure A-1 and A-2). Packaging of drinks in polyethylene film (oxygen permeable) resulted in extremely rapid destruction of the AA and DHAA. Experimental data and the results reported from a study done by Dennison and Kirk, (1978) indicated that oxygen must be evaluated as a reactant in the stability of AA. Figure 17 shows the effect of increasing amino acids concentration at the highest AA content (71.8 mg/100 ml) in samples stored in retort pouches. There was a rapid decrease in AA concentration with the first 2 to 4 weeks storage, followed by a slower decrease for the remainder of the 20 weeks storage period. Such an initial rapid loss may be due to oxidation by residual oxygen in the drinks reacting with AA. After this initial period, AA was probably degraded anaerobically, at rate lower than by aerobic process. Considerable loss of AA occured in the presence of the highest level of amino acids (1.26%). This suggests that amino acids may have some effect on the AA degradation.

In samples containing 1.26% amino acids, AA retention was reduced to 56% of its initial level after 20 weeks.

Samples containing 0.66 and 0.06% amino acids retained 61 and 63% respectively, after the same period of time. Figure 18 shows the same effect but with a fixed concentration of 38.0 mg/100 mL of AA. It is interesting to note that this level of AA is similar to that found in single strength orange juice. At this level of AA the increase of amino acids from 0.06 to 0.66% did not affect the loss of ascorbic acid. The per cent losses in single strength orange juice stored in retort pouches were close to those in orange drinks 4, 5, 7, and 8 which contained the low levels of amino acids. Curl et al., (1949) working with ascorbic acid-amino acids-sugar systems, reported similar results and suggested that, since the losses in orange juice were of same order of magnitude as the model systems, it is possible that the reactants involved are the same or similar.

Under similar conditions, but with an initial concentration of 4.2 mg/ 100 ml ascorbic acid, and increasing amino acids (Figure 19), there was no significant effect of amino acid levels on AA retention. The small amount of AA present (4.2 mg/100 mL) in these drinks made any difference difficult to detect.

Dehydroascorbic Acid Production

All AA initially added to the orange drinks was in the reduced form. However, our results showed that significantly high levels of DHAA were found in all mixtures at the zero time analysis. These unusually high levels of DHAA are the result of incorporation of air and oxidation of a large amount of AA during preparation of the drinks.

During the first 2 weeks the levels of DHAA decreased rapidly in all mixtures. This decrease reflects either the conversion of DHAA to DKGA, or its reaction with amino acids, we were unable to measured DKGA with the HPLC procedure used. It has been reported that DHAA reacts very rapidly with alpha amino acids to produce strongly colored (reddish to brown) complexes (Koppanyi et al., 1945). Once DHAA is degraded, its antiscorbutic activity is lost. In samples stored in retort pouches (Figure 17 and 18) the amount of DHAA gradually increased after 8 weeks and remained relatively constant at this level. These results are in agreement with those reported by Moore et al. (1944) who showed that the levels of DHAA in canned and bottled orange and grapefruit juices stored for six months at 4 and 27°C remained constant at about 1-2% of the total ascorbic acid, neither container type nor storage temperature appeared to influence the DHAA level. Smoot and Nagy (1980) reported that with single strength grapefruit juice stored at different temperatures the DHAA and DKGA contents remained virtually unchanged during a 12 week period. Our results showed that DHAA, following an initial drop in concentration, remained relatively constant throughout the 20 week storage period. No accumulation of DHAA occurred in the different orange drinks and once it is formed it is further transformed to DKGA or reacts with amino acids. Although AA-induced browning in citrus products, which involves its conversion to furfural, is well known (Braverman, 1963), discoloration involving the reaction of amino acids with DHAA or DKGA has not been widely investigated. Dulkin and Friedemann (1956) studied the role of DHAA in the browning reaction and reported that

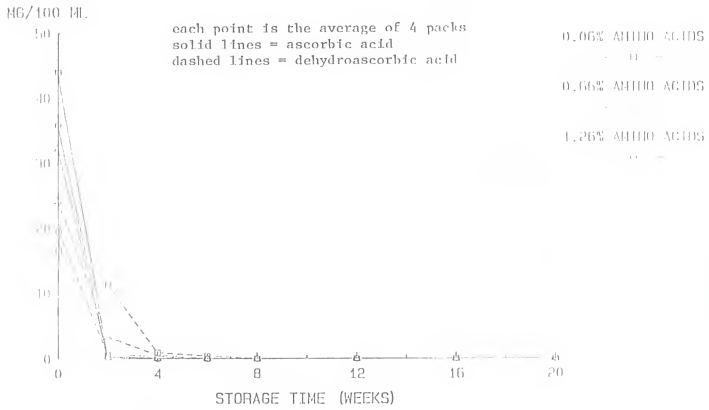

Figure 16. Effect of Amino Acid Concentration on Ascorbic Acid Retention. (initial level of ascorbic acid 71.8 mg/100 ml, samples stored in polyethylene pouch)

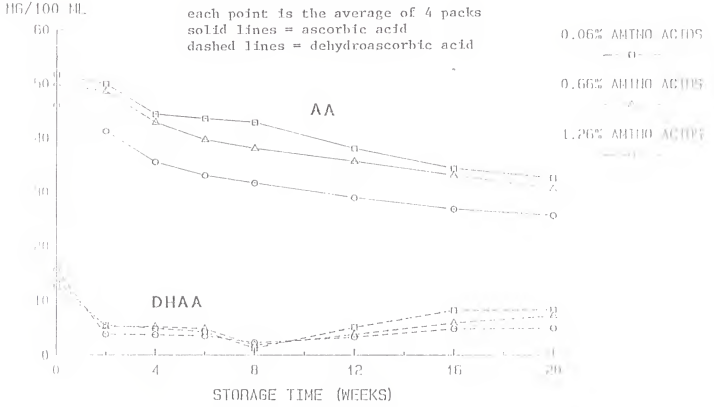

Figure 17. Effect of Amino Acid Concentration on Ascorbic Acid Retention. (initial level of ascorbic acid 71.8 mg/100 ml, samples stored in retort pouch)

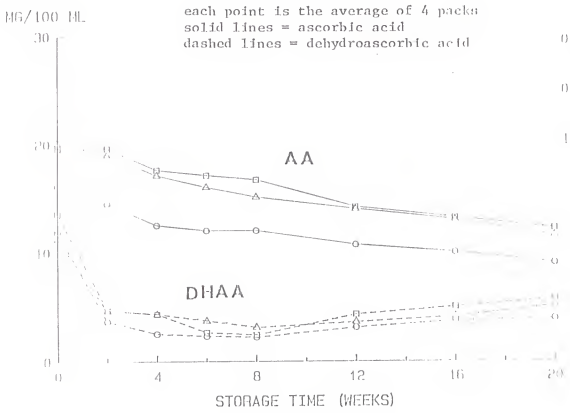

Figure 18. Effect of Amino Acid Concentration on Ascorbic Acid Retention. (initial level of ascorbic acid 38.0 mg/100 ml, samples stored in retort pouch)

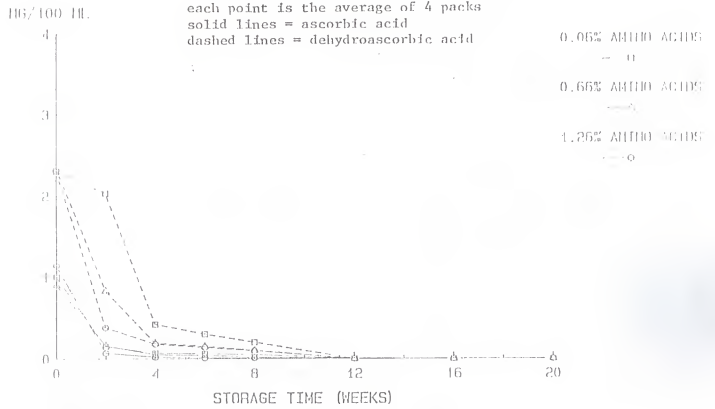

Figure 19. Effect of Amino Acid Concentration on the Ascorbic Acid Retention. (initial level of ascorbic acid 4.2 mg/100 ml samples stored in retort pouch)

oxidation of AA to DHAA is a prerequisite to browning. They also observed that the rate of browning was not increased by the addition of an amino acid (tryptophan) and suggested that browning in their experiment was not identical to the Maillard-type of reaction, and that DHAA must first undergo an irreversible transformation to DKGA. This transformation was first postulated by Herbert et al. (1933) and was substantiated by the work of Penney and Zilva (1943).

Browning As Influenced By Ascorbic Acid Concentration

Since AA appeard to be the most reactive constituent in the orange drinks investigated, and since browning in citrus juices is considered to be mainly due to AA degradation, it is therefore reasonable to expect the trend in browning to correspond to that of AA loss. Browning in the different orange drinks, as measured by absorbance and as affected by ascorbic acid concentration is presented in Figures 20, 21, and 22. In all cases, the diffusion of atmospheric oxygen through the polyethylene film was found to decrease significantly the ascorbic acid retention and to increase the development of brown pigments of non-enzymatic origin. It is therefore reasonable to conclude that the brown pigments originate from the oxidation of ascorbic acid. Orange drinks containing an initial concentration of 0.06% amino acids and with increasing level of AA (Figure 20) showed little browning in samples stored in retort pouches. However, in the presence of oxygen there was significant darkening. The effect of increasing AA was more significant in the presence of oxygen than in its absence. Under similar conditions, but with a initial concentration of 0.66% amino acids and increasing level

of AA (Figure 21) there was a significant effect of AA concentration only in the presence of oxygen. High AA concentrations yielded a darker colored product than the low AA level. It is interesting to note that since 0.66% amino acids is the concentration range found in orange juice then an increase of the AA concentration in the absence of oxygen would not increase the browning significantly whereas, in the presence of oxygen, there would be significant darkening. This result indicates that oxidation of ascorbic acid is a major factor in the formation of brown pigments.

Orange drinks containing 1.26% (w/w) amino acids and increasing level of AA (Figure 22) showed significant differences in browning. In both packaging materials AA had an effect on the browning. In the polyethylene pouch, with high oxygen permeability increases in browning followed increases in the ascorbic acid content, whereas there was not a linear relationship in the retort pouch. In retort poch high levels of ascorbic acid (30.8 , 72.8 mg/100 ml) increased the browning. This is in agreement with the earlier finding that only when the level of amino acids was high (1.26%) in retort pouch was the ascorbic acid retention decreased. This indicates that high amino acid levels accelerated ascorbic acid degradation which resulted in an increase in browning.

Browning As Influenced By Amino Acids Concentration

Orange drinks containing 4.2 mg/100 ml of ascorbic acid and increasing levels of amino acids (0.06, 0.66, 1.26 %) browned only slightly even in the polyethylene pouch (Figure 23). Increasing the concentration of amino acids at the low level of AA had no significant

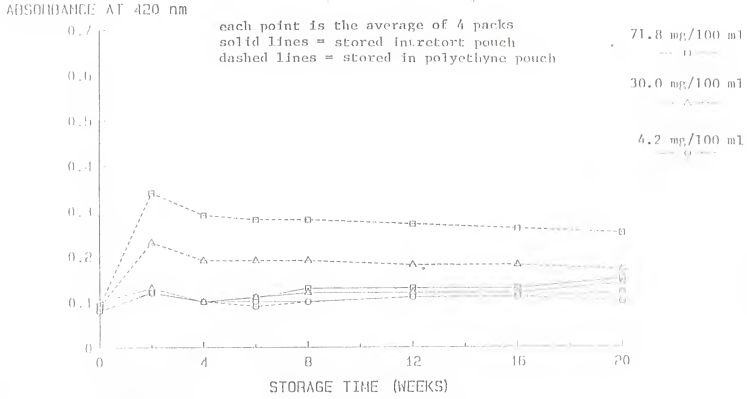

Figure 20. Effect of Ascorbic Acid Concentration on Browning of Orange Drinks with 0.06% Amino Acids

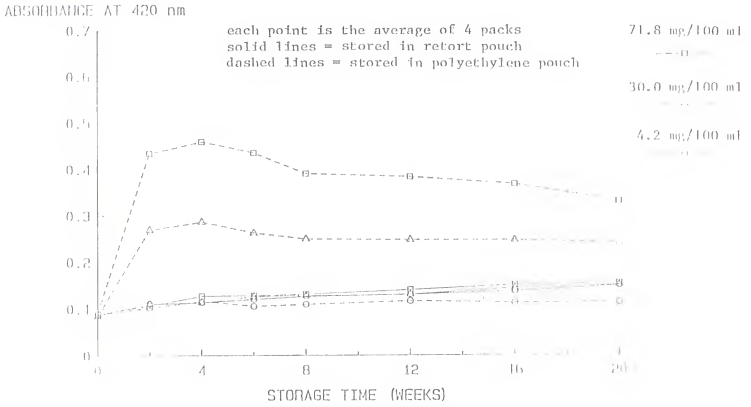

Figure 21. Effect of Ascorbic Acid Concentration on Browning of Orange Drinks with 0.66% Amino Acids

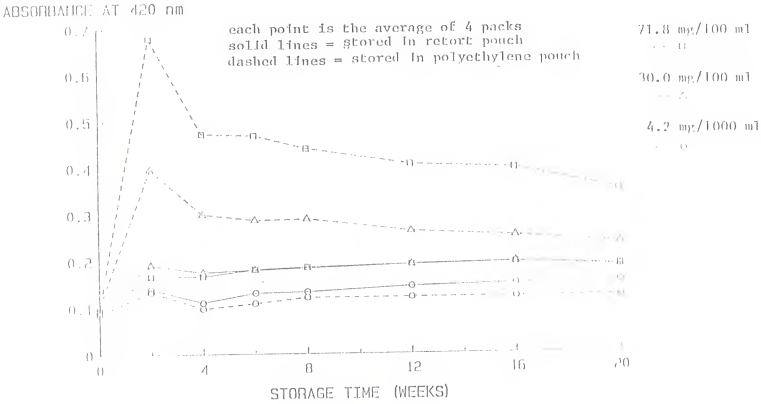

Figure 22. Effect Of Ascorbic Acid Concentration on Browning of Orange Drinks with 1.26% Amino Acids

effect on the browning of the product. This indicates that the browning in this case is probably different from the Maillard reaction. Furthermore, it is well known the conditions which would favor the development of the Maillard reaction, near neutral pH or slightly alkaline pH, are absent in orange juice. Therefore, it is unlikely that this mechanism is the major contributor to the browning of a highly acid product such as orange juice at pH 3.8. Under similar conditions but with a higher level of AA (Figures 24 and 25), there was an appreciable increase in the darkening only at 1.26% amino acid concentration. This effect was more significant in the presence of oxygen than in its absence. It is interesting to note that when samples were stored in retort pouches an increase of amino acids from 0.06 to 0.66% had no significant effect on the browning regardless of the levels of ascorbic acid. These results indicate, that in orange juice where the amino acid concentration is below 0.66%, the amino acids will not significantly affect the browning of the product. Joslyn and March (1935) reported that amino acids play a minor role in the oxidative nonenzymatic browning of orange juice; this has been confirmed for orange juice and for a model system composed of ascorbic acid and glycine and other amino acids (Dulkin and Friedemann, 1956; and Joslyn 1957). Our study further substantiated this finding. The accelerated breakdown of ascorbic acid in the presence of high levels of amino acids has been reported in the literature (Joslyn, 1957; Clegg, 1964; and Seck and Crouzet, 1981). On the basis of the chemical structure of ascorbic acid, it has been postulated that in citrus juice, ascorbic acid reacts with amino acids in much the same

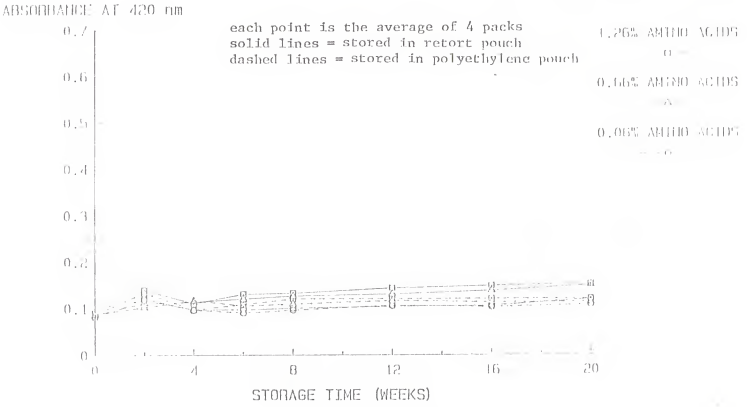

Figure 23. Effect of Amino Acid Concentration on Browning of Orange Drinks (4.2 mg/100 ml AA)

way as sugars react in the Maillard browning system (Joslyn, 1957). The fact that no significant browning occurred between 0.06 and 0.66% amino acid concentration in orange drinks, in the absence of oxygen, suggests that ascorbic acid must first be oxidized to dehydroascorbic acid. Hodge (1953), Clegg (1964), and Kurata et al. (1973) reported that in the presence of amino acids, it is dehydroascorbic acid which is the reactive intermediate in the pathway to furfural and brown pigment production, probably through the Strecker degradation of amino acids as suggested by Reynolds (1965). However, it is possible also that some of the active groups resulting from oxidation of ascorbic acid were polymerizing and not involving amino acids. This is demonstrated by the similar degree of browning in samples containing the same levels of ascorbic acid but different levels of amino acids.

It is interesting to note that the intensity of browning under aerobic conditions increased to a maximum and then decreased. The more rapid the initial pigment production was, the more definite was this decrease. Reaction in the retort pouches proceeded so slowly that no maximum was reached during the time the reaction was followed. Our results are in agreement with those of Seaver and Kertesz (1946) who found maximum color production when AA was heated in the presence of glycine, and with Lahikainen et al. (1958) who reported the intensity of browning under aerobic condition to increase to a maximum then decreased.

Joslyn (1957) reported that the concentration of AA in browning systems determined whether or not the color production went through a maximum. At low concentration of AA the color increased continuously

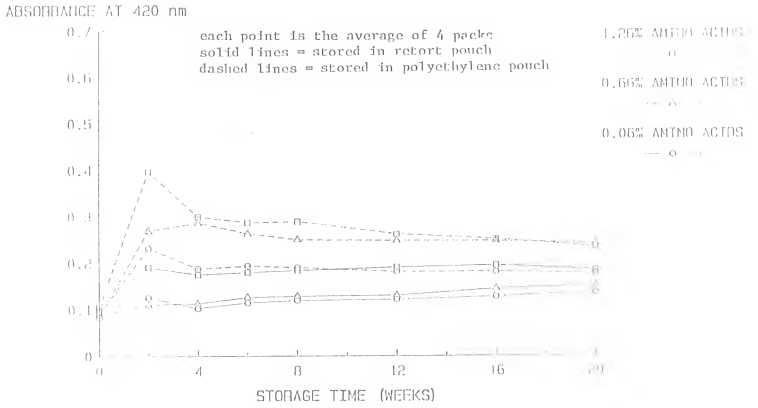

Figure 24. Effect of Amino Acid Concentration on Browning of Orange Drinks (38.0 mg/100 ml AA)

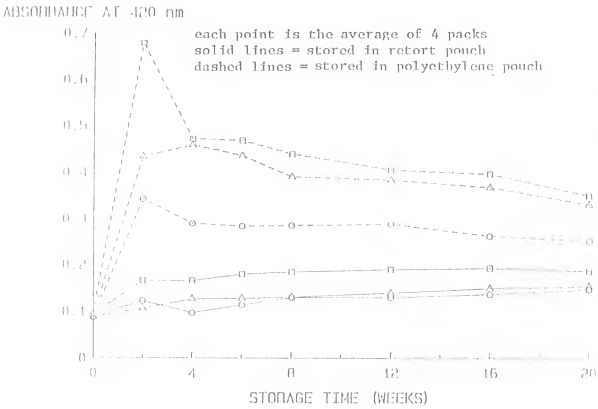

Figure 25. Effect of Amino Acid Concentration on Browning of Orange Drinks (71.8 mg/100 ml AA)

with time. The same relation was observed at high concentrations of AA but at intermediate levels the color production went through a maximum. This bleaching phenomena may be due to further changes in the degree of polymerization or/and to the precipitation of the brown pigments formed. With regard to the second possibility, considerable dark precipitate was observed in samples stored in polyethylene pouch

Statistical Analysis

Because of the possible interaction between the different factors involved in the browning of the orange drinks, a statistical analysis of the data was conducted to determine the significance of these interactions at different storage times. Table 11 shows the results

Table 11

Three-Factor Analysis of Variance of the Browning Data at 8 weeks.

Source	df	SS	MS	F
Oxygen	1	0.1881	0.1881	62.7
AA	2	0.2514	0.1057	35.2
aa	2	0.0636	0.0411	13.7
Oxy*AA	2	0.1687	0.0963	32.1
Oxy*aa	2	0.0075	0.0125	4.1
AA*aa	4	0.0108	0.0040	1.3
Error	4	0.0091	0.0030	
Samples within	54	0.0063	0.0001	
Total	71	0.7057		

df= degrees of freedom; SS= sum of squares; MS= mean square
F= MS factor/MS error; AA= ascorbic acid; aa= amino acids.

of the 3-factor analysis of variance for browning data at 8 weeks. An examination of the F values indicates that the main effects of oxygen, ascorbic acid, and amino acids are statistically significant.

Another important observation drawn from this table is the significance of the two-factor interaction (Oxygen*AA). Therefore, the analysis of variance for the polynomial models for AA was conducted for each type of packaging material. Considering, there are two quantitative factors (ascorbic acid and amino acids) with equally spaced levels, each factors'effect can be partitioned into 2 polynomial effects, namely a linear effect and a quadratic effect. Figure 26 summarizes the effects of ascorbic acid and amino acids on the browning of orange drinks at 8 week storage. Since the interaction AA*aa was not significant, the effect of ascorbic acid at the 3 levels of amino acids were combined and are presented at each level of oxygen. For amino acids, the effect of both types of packaging and at the different ascorbic acid concentrations were combined. The results indicate that in the retort pouch an increase in ascorbic acid concentration did not affect the browning of orange drinks, whereas in the polyethylene pouch browning increased linearly with increasing ascorbic acid concentration. The effect of amino acids was stricly linear, and was less pronounced than that of ascorbic acid.

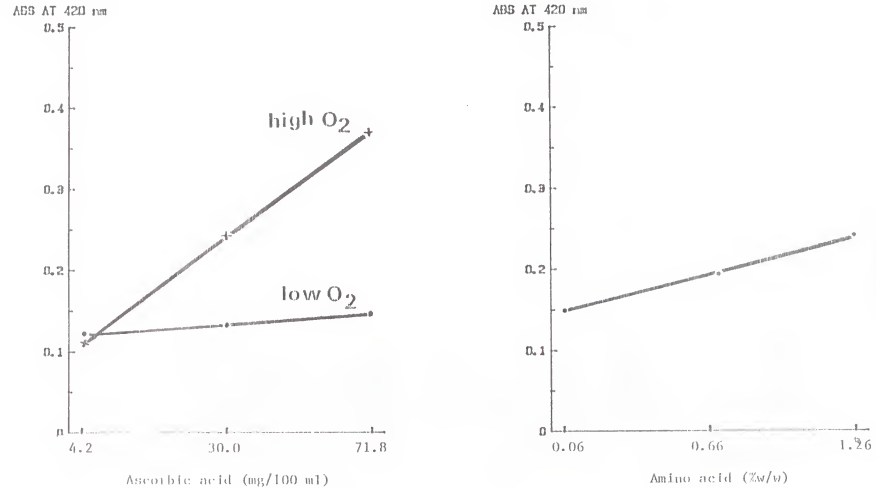

Figure 26. Effect of Ascorbic Acid and Amino Acid Concentration on Browning of Orange Drinks at 8 Week Storage

Conclusion

In conclusion, this study on orange drinks aseptically packaged and stored at room temperature has yielded additional data on nonenzymatic browning of the product. There were indications that:

1-The presence of oxygen greatly accelerated the loss of ascorbic acid and increased considerably the formation of browning pigments.

2-Considerable loss of ascorbic acid occurred during preparation and processing of the samples, due to incorporation of air.

3-High levels of dehydroascorbic acid were found in all samples at the zero time, indicating that there was considerable oxidation of ascorbic acid during preparation and processing of the samples.

4-Considerable loss of ascorbic acid occurred in the presence of amino acids, indicating that amino acids may have some effect on the degradation of ascorbic acid.

5-When samples were stored in retort pouches, the level of dehydroascorbic acid, decreased rapidly during the initial stage of storage, and remained relatively constant thereafter. This is probably due to the reaction of DHAA with amino acids to produce browning pigments.

6-Ascorbic acid was the most reactive constituent of orange drinks, with respect to the formation of browning pigments. Its effect on the browning reaction was much more intense in the presence of oxygen.

7-Amino acids have an effect on the browning of orange drinks. This effect was stricly linear within the concentration range used, and was more pronounced in the presence of high levels of ascorbic acid.

EFFECT OF AMINO ACID CONCENTRATION, PROCESSING AND STORAGE CONDITIONS ON THE QUALITY OF ASEPTICALLY PACKAGED ORANGE JUICE AND ORANGE DRINKS.

Introduction

To obtain additional information on the relative reactivity of the major constituents believed to be involved in browning, a second study was conducted.

The objective of this study was to investigate the effect of amino acid concentration and deaeration vs. no deaeration on the quality of aseptically packaged single strength orange juice and orange drinks, when the packages are stored under aerobic and anaerobic conditions.

Material and Methods

Reagent

Frozen concentrate (Citrus World Inc., Lake Wales, Fla.), high fructose corn syrup (Pacific Gateway Co., San Francisco, CA.), ascorbic acid (Eastman Kodak Company, Rochester, N.Y.), amino acids (Ajinomoto U.S.A. Inc., Englewood Cliffs, N.J.), citric acid and potassium citrate (Pfizer Chemical, Inc., New York, N.Y.), sucrose (local market), FD&C Yellow #5, FD&C Yellow #6, and Redd natural Orange Juice Drink Flavor 375316U (Redd Citrus Specialities) were used to make the orange drinks.

Orange Drinks Composition

Three orange drink mixtures containing 10% orange juice, 38.0 mg/100 ml ascorbic acid and various amounts of amino acids were prepared, aseptically packaged, stored at controlled temperature set to 75°F, and examined periodically over a period of 16 weeks. The composition of the orange drinks is given in table 12. In addition, the orange drink mixtures contained 10% orange juice, 5% sucrose, 5% HFCS, 0.01% Redd natural orange juice drink flavor (375316U0), 0.0015% FD&C yellow # 5 and 0.0006% FD&C yellow # 6.
Glucose and fructose were provided by the HFCS which contained 71% solids, 42% fructose, 50% glucose, 5% disaccharides, and 1.5% maltose. A mixture containing equal amounts of arginine, aspartic acid, and 4-aminobutyric acid was used to make the drinks. The final degree Brix of the orange drinks was 12.

Table 12

Composition of Orange Drinks .

Products	Ascorbic acid mg/100 ml	Amino acids added w/w	other constituents
M 1	38.0	0.0%	All mixtures
M 2	38.0	0.4%	contained 0.6%
M 3	38.0	0.8%	citric acid and 0.2% potassium
M 0	38.0	S.S.O.J.	citrate

Preparation of the samples

The juices and drinks were aseptically filled into flexible packages, using the Tetra Pak, Inc. research pilot plant facilities in Irving, Texas. The packaging material is a laminate of: (from outside to food contat surface) polyethylene/printed paper/polyethylene/ aluminum foil/polyethylene/polyethylene. The aluminum foil gives the package the excellent barrier properties to both light and oxygen. The product contact surface of polyethylene provides an inert lining for the package and also is necessary for forming the hermetic under the proper conditions of temperature and pressure. The mechanics of the Brik Pak system can be divided into four functions:

1- Sterilization of the product

2- Sterilization of the packaging material

3- Forming and filling in sterile surrounding

4- Sealing of packs to prevent recontamination

In the Ultra High Temperature (UHT) processing the product was rapidly heated to 195°F and held for 10 seconds. The juice was then cooled to room temperature and conveyed in a closed system to a vertical form-fill and seal machine, Model AB3.

This machine as described by Russo and Bannar (1981) starts with a reel of printed, laminated material web containing 2,000 to 22,000 packages which is loaded into a cassette in the back of the machine. After attachment to the machine, the web is drawn upwards to the top of the unit where it is stamped with the date and code number. A plastic longitudinal strip is applied to one edge of the paper. This strip has two functions: to reinforce the longitudinal seam, and to

prevent the product from coming in contact with paper edge. After application of this strip, the material passes through a peroxide bath where it is coated with a thin film of 35% hydrogen peroxide for chemical sterilization. A pair of pressure rollers removes surplus hydrogen peroxide which runs back into the sterile bath.

Passing a bending roller on the very top of the machine the packaging material starts its way down towards the front of the machine through forming rings which shape the material into a longitudinally sealed tube. The remaining hydrogen peroxide is evaporated. The product to be packed is introduced through a stainless steel filling tube which is jacketed by a second tube through which sterile hot air can be blown into the paper tube. As an initial stage in the sealing of the longitudinal seam, one edge of the packaging material passes through an element which is heated by hot sterile air. The longitudinal seam is sealed in this forming ring where both edges of the packaging material are pressed together.

The tube heater consists of a coil-shaped electric element which heats the inside of the packaging material with radiant heat. This radiant heat sterilizes the packaging material and at the same time a sterile atmosphere is created above the liquid level. The flow of product into the tube is modulated by a butterfly valve which in turn is controlled by a float. The level of product in the tube is approximatly two feet above the bottom transversal seal and is regulated mechanically by a float so that it is always higher than the mouth of the filling tube. By this means, frothing is avoided. Transversal seams are done at regular intervals below the level of the

product. In order to seal transversely, the product has to be squeezed away from the sealing zone. This is done by closing the sealing jaws, applying pressure and then heat. Individual units are cut at a rate of about one package per second. The pouches thus obtained are fed into the final folder, where they receive their brik like shape by sealing the flaps down onto the sides and the bottom of the package respectively.

To evaluate the effect of deaeration on the quality of aseptically packaged orange juice two types of products were obtained; one where the juice obtained was deaerated by vacuum. In the second the deaerator was turned off and the juice obtained was nondeaerated. All orange drinks were deaerated. The samples were then stored at 75°F under aerobic or anaerobic conditions as indicated in Table 13. For practical reasons, sensory evaluation was not performed on orange drinks M 2 and M 3 stored anaerobically.

Table 13

Processing and Storage Conditions of Orange Juice and Orange Drinks

Stge	Orange juice			Orange drink deaerated					
Time wks	OJD		OJND	M 1		M 2		M 3	
	ANA	AER	AER	ANA	AER	ANA	AER	ANA	AER
0	4	4	4	4	4	2	4	2	4
4	4	4	4	4	4	2	4	2	4
8	4	4	4	4	4	2	4	2	4
12	4	4	4	4	4	2	4	2	4
16	4	4	4	4	4	2	4	2	4

Immediately after processing samples to be stored anaerobically were placed into BBL GasPak jars (150 mm) where the anaerobic atmosphere (H_2 and CO_2) was provided by BBL GasPak anaerobic systems (Becton Dickinson and Co. Cockeysville, MD). The samples were then transported to the University of Florida, Gainesville for storage and analysis. One day after arrival the anaerobic samples were transferred to 2 large glass containers where air was removed using a vacuum pump and was replaced with a nitrogen atmosphere. The anaerobic conditions were controlled through a GasPak Disposable Anaerobic Indicator, and nitrogen was continously flushed as needed. All analytical measurements were made on 3 replicate packages and the coefficient of variation was small.

Methods of Analysis

Ascorbic and Dehydroascorbic Acids

Ascorbic and dehydroascorbic acids were determined using the procedure of Kacem et al., (1986).

Browning

Production of browning pigment expressed as absorbance at 420 nm, was measured spectrophotometrically using the method of Meydav et al., (1977).

Amino acids analysis

Amino acids were measured using a Beckman amino acid analyzer Model 119 CL, equipped with a 6x460 mm column and W3 resin with a bed height of 220 mm. The buffer and ninhydrin flow rates were 44 and 22 ml/h respectively. The recorder chart speed was 6 in/h. The amino acid analyzer was connected to a 3390 A Hewlett-Packard integrator.

Sensory evaluation

Sensory evaluation (color and flavor) of samples was conducted at various times during storage by at least 15 inexperienced taste panelists from among department personnel, using a 1 to 9 hedonic scale (9 being the best) to establish general acceptability of the products.

Statistical analysis

Regression methods were used for the calculation of factor effects, and for the analysis of variance (ANOVA).

Results and Discussion

Orange Juice

Ascorbic acid and dehydroascorbic acid concentrations. Figure 27 and Figure 28 show ascorbic acid and dehydroascorbic acid retentions as a function of storage time. An examination of these plots reveals that there were some differences among the 3 juices. The nondeaerated juice showed the largest loss of ascorbic acid. The anaerobically stored juice, had better ascorbic acid retention than the aerobically stored juice. At the end of the 16-week storage period, the retention of ascorbic acid in orange juice deaerated (OJD) anaerobically stored, OJD aerobically stored, and orange juice nondeaerated (OJND) were, 97, 91, and 86% respectively. The fact that the nondeaerated juice showed the highest drop in ascorbic acid content during the first 4 weeks of storage indicates the importance of oxygen in ascorbic acid degradation. After this period, retention of ascorbic acid in both types of juices under the same storage conditions were practically parallel.

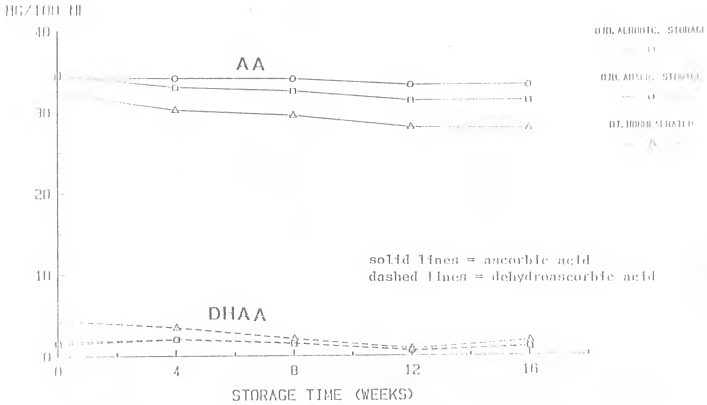

Figure 27. Ascorbic Acid and Dehydroascorbic Acid Concentrations in Orange Juice as Influenced by Processing and Storage Conditions

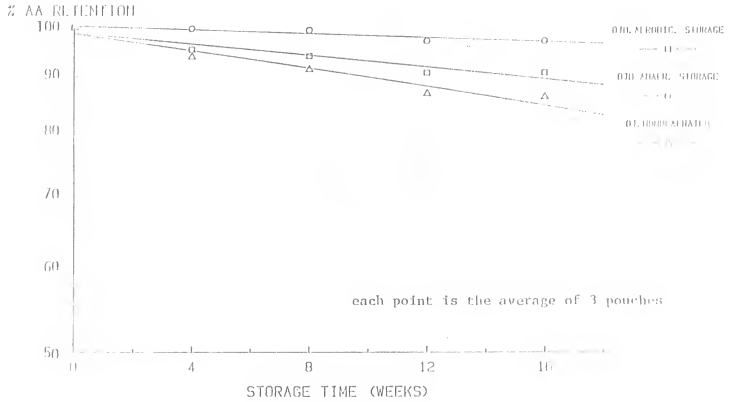

Figure 28. Ascorbic Acid Retention (Log scale) in Orange Juice as Influenced by Processing and Storage Conditions

Deaerated and anaerobically stored juice exhibited the highest ascorbic acid retention value. This result suggests that some oxygen was transmitted through the package seal area under aerobic conditions. The heat seal area, generally polyethylene or polypropylene, may permit some gas exchange.

No significant differences in the concentration of dehydroascorbic acid were noted between the juices. The concentration of DHAA remained practically constant during the storage period except for the nondeaerated juice which showed a slightly higher concentration during the first 4 weeks. Figure 28 illustrates the loss of ascorbic acid potency (log scale) as a function of time. The retention of ascorbic acid followed a first order kinetic model since the data fit a straight line. It is possible that the reaction is pseudo first order. Studies have shown ascorbic acid degradation to be first order (Huelien, 1953; Waletzko and Labuza, 1976; Saguy et al., 1978; Passy and Mannheim, 1979). From this plot the rate constants of ascorbic acid destruction (K) were determined for OJD anaerobically stored, OJD aerobically stored, and OJND and were 0.84, 2.32, and 3.49 weeks^{-1}, respectively.

Browning. Changes in color during storage are presented in Figure 29. No significant difference in browning could be observed in the three types of juices. The limited browning could be due to the fact that insufficient ascorbic acid was degraded (86-97% retention). Curl and Talburt (1961) found that browning in citrus juices could be observed only after a 10 to 15% decrease in ascorbic acid content had taken place. The absorbance increased with storage time, but this

increase took place only after an initial lag period (Joslyn, 1957; Karel and Nikerson, 1964). During this lag period, colorless compounds are probably formed which do not contribute to an increase in absorbance. Joslyn and Marsh, (1935) and Joslyn et al., (1934) found in Valencia orange juice that storage at elevated temperature does increase the rate of browning, and darkening began to occur when the iodine reducing value of the juice had decreased from 26.0 to 12.9. They believed that reducing substances must be almost completly destroyed before browning can begin. Hamburger and Joslyn (1941) suggested that the darkening occurs after the ascorbic acid is in the dehydroascorbic acid form and when no readily oxidizable substances are left in the juice.

Sensory evaluation. Sensory evaluation of the juices was performed using the hedonic scale. As flavor was judged by taste, it cannot be presented as a sharply defined analytical result. Figure 30 shows the mean sensory scores for the three juices as a function of storage time. In general, all samples of the juices showed slight change in flavor. The mean value for all samples at zero time was 7.2 (like moderately), and at 16 weeks was 5.9 (like slightly). Each 4 week time period was subjected to an analysis of variance and there was no significant difference at any time period among the three juices at a 95% probability level; and at the end of the 16 weeks storage period, the different orange juices were considered equal in flavor, with mean scores of 5.6, 6.0, and 5.4, respectively for anaerobically, aerobically stored and nondeaerated orange juices.

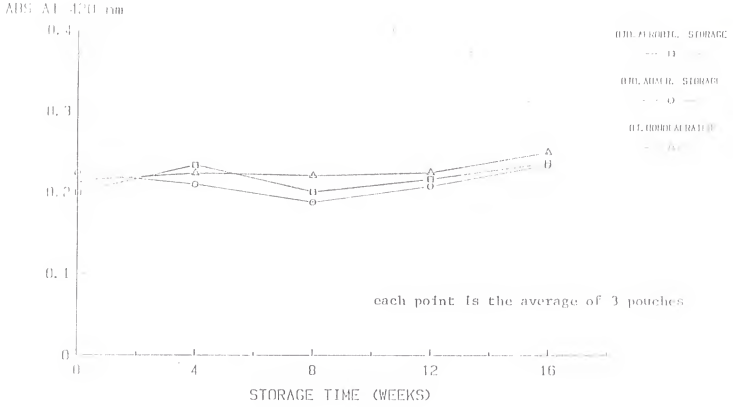

Figure 29. Browning Formation in Orange Juice as Influenced by Processing and Storage Conditions

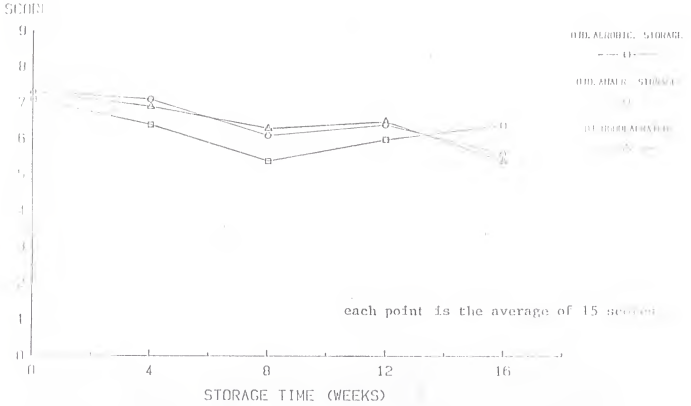

Figure 30. Flavor of Orange Juice as Influenced by Processing and Storage Conditions

Orange Drinks

Ascorbic and dehydroascorbic acids. The degradation of ascorbic and dehydroascorbic acids in aseptically packaged orange drinks, stored under different conditions was studied as a function of amino acid concentration and storage time. The experimental results for the loss of ascorbic acid are presented in Figure 31 and Figure 32. Each ascorbic acid and dehydroascorbic acid value is the average for three packages. These data (Figure 32) conformed to the first-order function

$$\frac{d(C)}{dt} = K(C)$$

where (C) = molar concentration of ascorbic acid

t = time (weeks)

K = first-order rate constant ($weeks^{-1}$)

The experimentally determined rate constants for ascorbic acid degradation in the different orange drinks are presented in Table 14. Examination of these data showed a significant increase in the rate constants of ascorbic acid loss with the increase of amino acids level from 0.4% to 0.8%. When the amino acids level was increased from 0.0 to 0.4 the rate constant did not increase significantly. These results are in agreement with our previous experiment when amino acids significantly affected ascorbic acid degradation only when added at the 1.26% level.

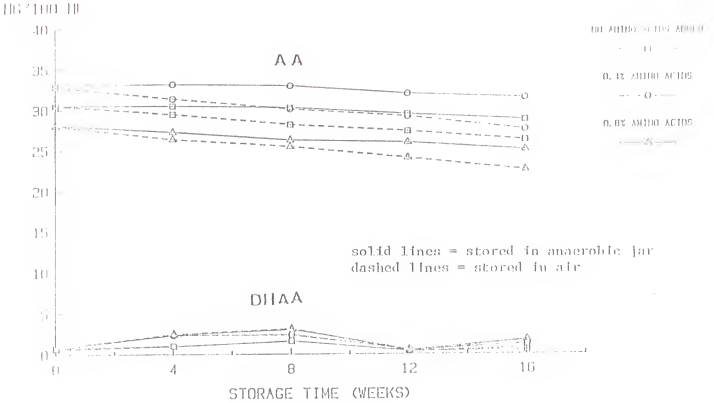

Figure 31. Ascorbic Acid and Dehydroascorbic Acid Concentration of Orange Drinks as Influenced by Amino Acid Content and Storage Conditions

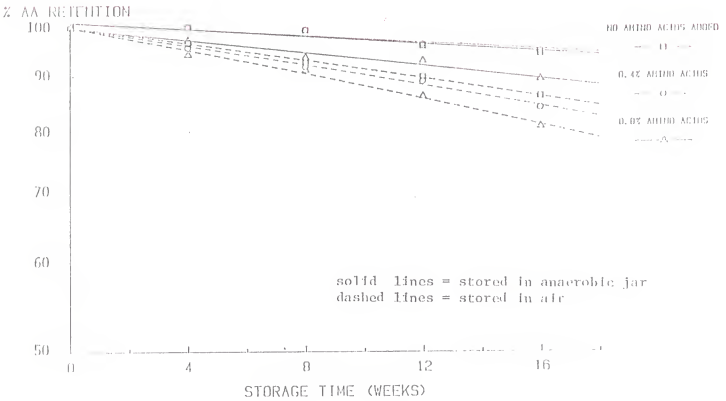

Figure 32. Ascorbic Acid Retention (Log scale) in Orange Drinks as Influenced by Amino Acid Content and Storage Conditions

Table 14

Rate Constants ($weeks^{-1}$) for Ascorbic Acid Loss as Function of Amino Acid Content and Storage Conditions in Orange Drinks

Amino acids added (%)	Storage conditions	
	Anaerobic	Aerobic
0.0	1.49	3.45
0.4	1.35	3.85
0.8	2.56	4.67

In general, ascorbic acid losses in samples stored under aerobic conditions were greater than in the samples stored under anaerobic conditions. At the end of the 16 weeks storage period the retention of ascorbic acid in orange drinks M1, M2, and M3 was 94.4, 94.9 and 89% for samples stored anaerobically and 86, 84, and 81% for samples stored aerobically.

Diffusion of oxygen through the package seal and the high sensitivity of ascorbic acid to oxygen appear to be the main reasons for this difference. Considerable loss of ascorbic acid occurred in the presence of the highest level of amino acids (0.8%) but no difference occurred when the amino acid level was increased from 0 to 0.4%. This is in agreement with our previous results, and indicates that high levels of amino acids affect the ascorbic acid degradation.

Browning. One of the main reasons for the reduction in the commercial value of citrus products is the nonenzymatic browning. The data for the browning expressed as absorbance at 420 nm and as a

function of amino acid content are given in Figure 33. No detectable browning occurred in any of the orange drinks. The absorbance measurement remained almost constant for the 16 week storage period. This delay in color changes, also observed by Karel and Nickerson (1964), was referred to as the time during which colorless intermediates of the browning reaction were formed (Clegg and Morton, 1965). The zero time difference in absorbance between the orange drinks is probably due to experimental error in the initial drinks composition.

Amino acids. Amino acid content in the orange drinks and as a function of storage time are given in Table 15 and Table 16. No detectable difference was observed during the 16 week storage. The amino acid concentration remained almost constant during the entire storage time. This result is in agreement with that of Joslyn and Marsh (1935) who observed that the amino-nitrogen level remained practically constant during the course of browning of Valencia and Navel juices, even after 126 days of storage at room temperature, even though the juice had become very dark brown in color.

Flavor. Flavor scores were not significantly different among the different orange drinks. At 16 weeks the mean flavor scores were 5.7 and 6.1 for orange drink M1 stored anaerobically and aerobically respectively. For orange drinks M2, and M3 the mean flavor scores were 5 and 5.2 respectively, and were not significantly different.

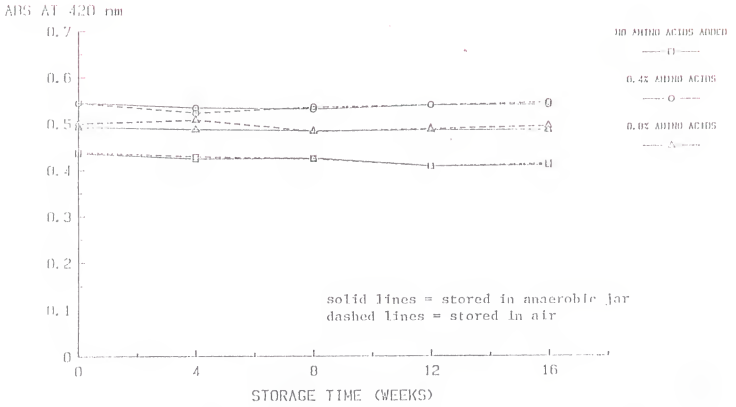

Figure 33. Browning Formation in Orange Drinks as Influenced by Amino Acid Content and Storage Conditions

Table 15

Changes in Amount of Amino Acids in Orange Drink M2 and M3 as Influenced by Storage Time (mg/100ml)

Amino acid	Storage conditions	storage time (weeks) 0	4	8	12	16
		M2				
Aspartic acid	Ana	129	105	122	127	120
	Aer	127	107	122	122	123
Arginine	Ana	172	170	173	180	177
	Aer	172	175	175	183	170
4-aminobutyric acid	Ana	140	138	135	139	140
	Aer	137	140	129	138	138
		M3				
Aspartic acid	Ana	191	168	203	210	190
	Aer	209	165	208	195	195
Arginine	Ana	257	241	253	268	245
	Aer	263	256	251	265	252
4-aminobutyric acid	Ana	241	240	242	247	240
	Aer	252	246	238	240	245

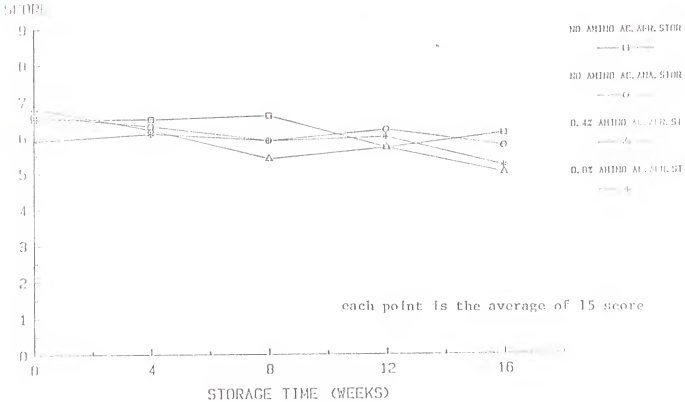

Figure 34. Flavor Changes of Orange Drinks as Influenced by Amino Acids Content and Storage Conditions

Conclusion

This study on single strength orange juice and synthetic orange drinks containing 10% orange juice, resulted in the following findings

1-Deaeration of orange juice resulted in increased retention of ascorbic acid, and had no effect on sensory quality of the juices.

2-Anaerobically stored juice had greater ascorbic acid retention than aerobically stored juice. However, there was no significant difference in sensory quality and browning between anaerobic and aerobic storage.

3-There was no significant difference in dehydroascorbic acid in orange juice and orange drinks due to storage conditions or deaeration.

4-There was no effect of amino acid concentration, anaerobic or aerobic storage on flavor by sensory evaluation orange juice or orange drinks.

5-The amino acid concentration of orange drinks remained constant during the entire storage period.

6-Increasing the amino acid concentration of orange drinks from 0.4 to 0.8% increased the ascorbic acid loss.

SUMMARY

The study on single strength orange juice and synthetic orange drinks containing 10% orange juice evaluated the effect of ascorbic acid, amino acids and oxygen permeable versus non permeable packaging. The effects of deaeration and anaerobic storage versus aerobic storage for orange juice aseptically packaged in commercial Tetra Pak flexible carton and stored at 75°F were also evaluated. Oxygen and ascorbic acid were found to be the most critical factors. Amino acids played an important role only in the presence of oxygen. The diffusion of atmospheric oxygen through the polyethylene film increased significantly the ascorbic acid degradation and the brown pigments formation. It is therefore, reasonable to conclude that the brown pigments in orange drinks originate from the oxidation of ascorbic acid.

High level of amino acids (1.26%) increased ascorbic acid loss in samples stored in the retort pouch (zero permeability to oxygen), and resulted in an increase in browning. The results indicate the possibility of at least two mechanisms for the formation of browning pigments depending on whether or not oxygen is present. Under the actual storage practice used in the aseptic packaging and because of the high acidity (pH = 3.8), it appears that of the three possible modes of browning, the ascorbic acid theory seems to be likely to occur in orange juice and orange drinks. The Maillard reaction plays

a minor role. This is in agreement with Joslyn and Marsh, (1935) who showed that the removal of sugars from orange juice had no effect on the browning of the product. However, the reviews of browning reactions have, in general, emphasized the complexity of the subject and the lack of specific knowledge on the chemical reactions and intermediates involved.

Browning, of whatever type, is caused by the formation of unsaturated, colored polymers of varying composition. It should be possible to analyze the browning complex by identifying the individual reactions and studying each in model systems, and determine which of these routes are operative and to what extent one affects the other in a given food, under a given set of conditions. The isotopic tracer techniques seems a promising outlook for an acceptable solution. Unfortunately, even though the use of this technique was an original objective of this study, it had to be omitted for financial reasons.

APPENDIX

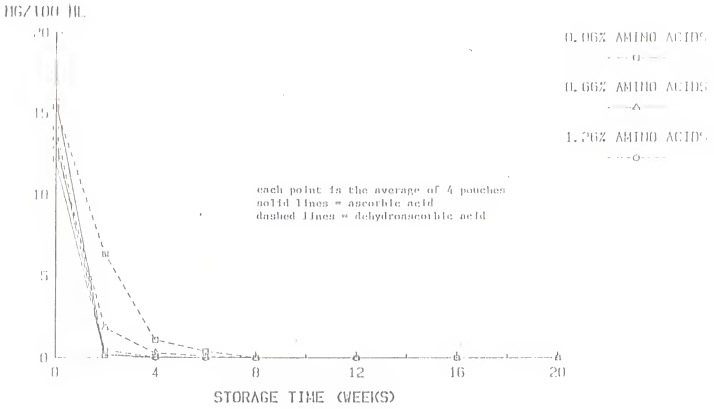

Figure A-1. Effect of Amino Acid Concentration on Ascorbic Acid Retention. (initial level of ascorbic acid 30.0 mg/100 ml, samples stored in polyethylene pouch)

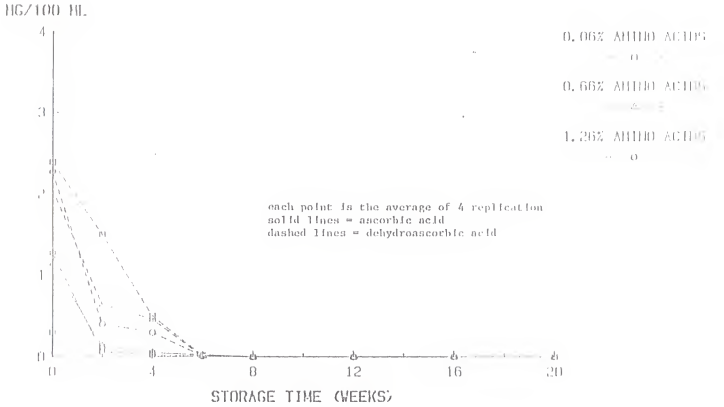

Figure A-2. Effect of Amino Acid Concentration on Ascorbic Acid Retention. (initial level of ascorbic acid 4.2 mg/100 ml, samples stored in polyethylene pouch)

Table A-1

Absorbance at 420 nm as a Function of Storage Time
(samples stored in retort pouch)

Pdt	Storage time (weeks)							
	0	2	4	6	8	12	16	20
M0	.205±2	.218±6	.242±9	.228±5	.250±5	.246±9	.260±6	.260±9
M1	.080±4	.118±6	.096±7	.096±1	.100±6	.104±2	.105±2	.119±9
M2	.086±9	.111±3	.113±8	.120±6	.126±2	.130±7	.137±2	.147±3
M3	.091±1	.139±8	.110±1	.130±9	.132±6	.143±5	.148±9	.152±9
M4	.098±9	.125±9	.102±4	.114±5	.119±5	.121±5	.127±9	.136±5
M5	.088±3	.110±3	.113±7	.126±4	.128±9	.130±9	.144±8	.150±3
M6	.107±6	.190±2	.174±7	.179±4	.183±3	.190±4	.194±4	.183±7
M7	.089±4	.123±5	.094±6	.114±9	.130±9	.130±9	.137±9	.147±9
M8	.088±7	.107±8	.127±9	.128±9	.131±9	.140±3	.150±8	.154±8
M9	.094±2	.166±9	.166±9	.180±3	.185±7	.190±9	.193±9	.186±9

[a]Number of pouches = 4. Mean $\pm$ SD($\times 10^3$)

Table A-2

Absorbance at 420 nm as a Function of Storage Time
(samples stored in polyethylene pouch)

Pdt	Storage time (weeks)							
	0	2	4	6	8	12	16	20
M0	.247±9	.298±8	.317±2	.317±8	.331±9	.323±1	.347±9	.314±9
M1	.083±7	.121±8	.096±9	.090±3	.096±2	.106±3	.102±7	.107±1
M2	.088±4	.102±5	.116±8	.105±7	.109±4	.116±9	.112±9	.113±4
M3	.087±5	.133±7	.097±9	.108±4	.120±2	.120±7	.120±9	.120±5
M4	.089±3	.231±4	.187±4	.193±1	.189±9	.180±6	.180±7	.175±9
M5	.084±6	.269±2	.286±7	.262±9	.249±5	.247±9	.246±8	.240±9
M6	.101±9	.396±8	.299±8	.286±5	.288±9	.261±9	.250±9	.233±9
M7	.087±5	.342±3	.289±1	.283±2	.285±9	.288±9	.262±9	.250±8
M8	.090±3	.434±5	.459±6	.435±8	.390±4	.383±6	.367±6	.330±5
M9	.093±7	.678±4	.472±9	.468±9	.439±8	.405±9	.396±9	.349±9

[a]Number of pouches = 4. Mean ± SD($x10^3$)

Table A-3

Ascorbic Acid Concentration as a Function of Storage Time
(samples stored in retort pouch)

Pdt	Storage time (weeks) 0	2	4	6	8	12	16	20
M0	31.5± .6	30.0± .5	29.0± .6	27.6± .6	26.5±.3	24.5±.9	22.0±.9	20.0±1.5
M1	1.0± .2	.2± .0	.1± .0	.1± .0	.1±.0	-	-	-
M2	.9± .3	.2± .0	.1± .0	.0± .0	.0±.0	-	-	-
M3	1.1± .1	.1± .0	.0± .0	.0± .0	.0±.0	-	-	-
M4	19.6± .3	19.6± .7	17.6± .6	17.1± .3	16.7±.1	14.2±.3	13.2±.5	12.2± .4
M5	20.1± .8	19.0± .2	17.1± .2	16.0± .5	15.1±.1	14.0±.8	13.0±.4	12.0± .8
M6	16.1± .9	14.5± .3	12.5± .2	12.0± .2	12.0±.3	10.7±.9	10.0±.9	9.0± .1
M7	51.6± .7	49.9± .9	44.3±2.4	43.5± .9	42.8±.9	38.0±.8	34.3±.8	32.5±1.5
M8	50.0±2.1	48.5±1.8	42.8±1.1	39.6± .9	38.0±.8	35.6±.5	33.0±.9	30.0±1.3
M9	46.0± .7	41.2±1.3	35.5± .7	33.0±2.0	31.6±.5	28.9±.8	26.8±.6	25.6±1.3

[a]Number of pouches = 4. Mean ± SD

Table A-4

Ascorbic Acid Concentration as a Function of Storage Time
(samples stored in polyethylene pouch)

	Storage time (weeks)							
Pdt	0	2	4	6	8	12	16	20
M0	30.6± .45	.3±.02	.2±.01	.1±.00	-	-	-	-
M1	.3± .02	.1±.00	.1±.00	.0±.00	-	-	-	-
M2	1.1± .01	.1±.00	.0±.00	.0±.00	-	-	-	-
M3	1.3± .23	.1±.01	.0±.00	.0±.00	-	-	-	-
M4	12.2±1.10	.1±.01	.1±.01	.0±.00	-	-	-	-
M5	15.9± .40	.2±.01	.0±.01	.0±.00	-	-	-	-
M6	13.4± .50	.1±.02	.0±.00	.0±.00	-	-	-	-
M7	44.0±1.7	.4±.06	.1±.09	.0±.00	-	-	-	-
M8	32.3±5.4	.3±.01	.1±.02	.0±.00	-	-	-	-
M9	35.8±1.2	.2±.01	.0±.00	.0±.00	-	-	-	-

[a]Number of pouches = 4. Mean ± SD

Table A-5

Dehydroascorbic Acid Concentration as a Function of Storage Time
(samples stored in retort pouch)

	Storage time (weeks)							
Pdt	0	2	4	6	8	12	16	20
M0	4.1± .1	2.9±.20	2.8±.50	2.7±.10	1.5±.30	2.3±.1	2.4±.2	2.5± .4
M1	2.3± .2	2.0±.40	.4±.02	.3±.02	.2±.05	-	-	-
M2	2.4± .2	.8±.06	.2±.07	.2±.05	.1±.04	-	-	-
M3	2.3± .1	.4±.03	.2±.09	.1±.03	.1±.03	-	-	-
M4	13.6± .4	4.7±.50	4.3±.60	2.6±.60	2.4±.20	4.3±.4	5.0±.9	5.7±1.1
M5	11.2± .4	4.6±.31	4.1±.41	3.7±.70	3.1±.20	3.6±.1	4.1±.3	5.1± .1
M6	11.9± .4	3.7±.20	2.5±.20	2.3±.10	2.2±.40	3.1±.3	3.7±.7	3.9± .8
M7	12.5± .1	5.5±.60	4.9±.20	4.2±.40	1.3±.10	5.1±.3	8.2±.1	8.3±1.7
M8	13.8± .2	5.2±.40	5.2±.50	4.8±.50	1.8±.10	3.8±.3	5.9±.3	7.2± .1
M9	15.6± .7	3.8±.10	3.7±.30	3.5±.20	2.3±.30	3.3±.4	4.8±.6	4.9± .5

[a]Number of pouches = 4. Mean ± SD

Table A-6

Dehydroascorbic Acid Concentration as a Function of Storage Time
(samples stored in polyethylene pouch)

Pdt	Storage time (weeks)							
	0	2	4	6	8	12	16	20
M0	5.30± .53	.85±.11	.90±.10	.05±.00	-	-	-	-
M1	2.39± .23	1.50±.00	.50±.06	.02±.00	-	-	-	-
M2	2.05± .04	.64±.02	.46±.02	.01±.00	-	-	-	-
M3	2.27± .14	.40±.02	.30±.01	.01±.00	-	-	-	-
M4	15.40± .86	6.34±.37	1.11±.10	.40±.00	-	-	-	-
M5	12.96± .84	1.84±.29	.30±.00	.10±.00	-	-	-	-
M6	14.12± .35	.46±.04	.05±.00	.02±.00	-	-	-	-
M7	16.50±1.10	11.34±.39	.97±.07	.40±.00	-	-	-	-
M8	24.10±2.90	3.37±.36	.54±.08	.05±.00	-	-	-	-
M9	19.46±1.00	.74±.04	.35±.10	.01±.00	-	-	-	-

[a]Number of pouches = 4. Mean ± SD

Table A-7

Absorbance at 420 nm as a Function of Storage Time
(samples stored in Tetra Pak carton)

Pdt[a]	Storage Time				
	0	4	8	12	16
OJDAE	.200±.003	.234±.005	.201±.003	.217±.005	.237±.003
OJDAN	.224±.005	.210±.006	.188±.004	.208±.005	.234±.009
OJND	.215±.007	.224±.010	.221±.005	.225±.009	.250±.012
OD1AE	.439±.012	.429±.005	.424±.009	.408±.005	.414±.002
OD1AN	.436±.023	.424±.009	.426±.007	.408±.008	.410±.007
OD2AE	.544±.007	.523±.012	.536±.008	.540±.004	.545±.004
OD2AN	.545±.019	.534±.015	.531±.006	.540±.001	.540±.009
OD3AE	.501±.026	.508±.023	.483±.004	.489±.004	.494±.008
OD3AN	.492±.031	.487±.018	.484±.003	.486±.009	.485±.005

[a] Number of pouches = 4. Mean ± SD

OJDAE = orange juice deaerated aerobically stored
OJDAN = orange juice deaerated anaerobically stored
OJND = orange juice nondeaerated
OD1AE = orange drink with no amino acids added, aerobically stored
OD1AN = orange drink with no amino acids added, anaerobically stored
OD2AE = orange drink with 0.4% amino acids, aerobically stored
OD2AN = orange drink with 0.4% amino acids, anaerobically stored
OD3AE = orange drink with 0.8% amino acids, aerobically stored
OD3AN = orange drink with 0.8% amino acids, anaerobically stored

Table A-8

Ascorbic Acid Retention (%) as a Function of Storage Time
(samples stored in Tetra Pak carton)

Pdt	Storage Time				
	0	4	8	12	16
OJDAE	100	95.3	94.0	90.7	90.7
OJDAN	100	99.7	99.4	97.1	97.1
OJND	100	93.8	91.3	86.7	86.7
OD1AE	100	96.7	92.5	89.8	86.2
OD1AN	100	100.0	99.3	96.0	94.4
OD2AE	100	95.7	91.8	89.0	84.1
OD2AN	100	100.0	99.4	96.4	94.9
OD3AE	100	94.2	91.1	86.1	80.7
OD3AN	100	97.1	93.6	92.9	89.3

OJDAE = orange juice deaerated aerobically stored
OJDAN = orange juice deaerated anaerobically stored
OJND = orange juice nondeaerated
OD1AE = orange drink with no amino acids added, aerobically stored
OD1AN = orange drink with no amino acids added, anaerobically stored
OD2AE = orange drink with 0.4% amino acids, aerobically stored
OD2AN = orange drink with 0.4% amino acids, anaerobically stored
OD3AE = orange drink with 0.8% amino acids, aerobically stored
OD3AN = orange drink with 0.8% amino acids, anaerobically stored

Table A-9

Flavor Score as a Function of Storage Time
(samples stored in Tetra Pak carton)

Pdt[a]	Storage Time				
	0	4	8	12	16
OJDAE	7.1±1.8	6.4±1.4	5.4±2.1	6.0±1.5	6.4±1.4
OJDAN	7.3±1.6	7.1±1.8	6.1±1.5	6.4±1.4	5.6±1.8
OJND	7.3±1.2	6.9±1.3	6.3±1.6	6.5±2.0	5.4±1.9
OD1AE	6.5±2.5	6.6±1.5	6.7±1.8	5.8±2.1	6.2±1.3
OD1AN	6.6±1.0	6.3±1.5	5.9±2.0	6.2±1.1	6.0±1.5
OD2AE	6.8±1.5	6.4±1.2	5.5±2.3	5.8±1.5	5.1±1.8
OD3AE	5.9±2.0	6.1±1.2	5.9±1.8	6.0±1.5	5.4±1.6

[a] Number of score = 15. Mean ± SD

OJDAE = orange juice deaerated aerobically stored
OJDAN = orange juice deaerated anaerobically stored
OJND = orange juice nondeaerated
OD1AE = orange drink with no amino acids added, aerobically stored
OD1AN = orange drink with no amino acids added, anaerobically stored
OD2AE = orange drink with 0.4% amino acids, aerobically stored
OD2AN = orange drink with 0.4% amino acids, anaerobically stored
OD3AE = orange drink with 0.8% amino acids, aerobically stored
OD3AN = orange drink with 0.8% amino acids, anaerobically stored

Table A-10

Sensory Evaluation Form

Name: ..... Date .....

Please evaluate these samples for flavor. Taste test each one. Use the appropriate scale to show your evaluation and check the point that best describes your feeling about the flavor of the sample.

Code_____	Code_____
.....Like extremely	.....Like extremely
.....Like very much	.....Like very much
.....Like moderatly	.....Like moderatly
.....Like slightly	.....Like slightly
.....Neither like nor dislike	.....Neither like nor dislike
.....Dislike slightly	.....Dislike slightly
.....Dislike moderately	.....Dislike moderately
.....Dislike very much	.....Dislike very much
.....Dislike extremely	.....Dislike extremely

Comments:

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BIOGRAPHICAL SKETCH

Bechir Kacem was born on December 24, 1948, in Ksar Hellal, Tunisia. After graduation from the Lycee Technique de Sousse, he attended the Faculte des Science at the University of Tunis, receiving a Bachelor of Science degree in chemistry in September 1973. He began his master's program in human nutrition in September 1974 at Harvard University. After graduation in June 1976, he went back to Tunisia and worked at the National Institute of Nutrition and Food Technology as an Assistant Professor for six years. He was admitted to the University Of Florida, Gainesville, in August 1982 to pursue graduate studies in the Food Science and Human Nutrition Department. He expects to receive the degree of Doctor of Philosophy in August, 1986.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

R. F. Matthews, Chairman
Professor of Food Science and
Human Nutrition

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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